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Proceedings of the Eighteenth Annual Aquatic Toxicity Workshop: September 30-October 3, 1991, Ottawa, Ontario

Comptes rendus du dix-huitième atelier annuel sur la toxicité aquatique: du 30 septembre au 3 octobre 1991, Ottawa, Ontario

Editors/Éditeurs

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Canadian Technical Report of Fisheries and Aquatic Sciences 1863

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PREFACE/PREFACE

The 18th Annual Aquatic Toxicity Workshop was held at the Government Conference Centre, Ottawa, Ontario, September 30-October 3, 1991. The Workshop included 3 plenary presentations, 98 platform presentations and 23 papers in poster sessions. Total attendance was 330.

The 18th Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshop in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal enchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are published with the support of the Department of Fisheries and Oceans.

Le 18e atelier annuel sur la toxicité a eu lieu le Centre de conférence du governement, Ottawa, Ontario les 30 septembre au 3 octobre 1991. Le atelier a donné lieu à 3 communications lors de séances plénières, 98 exposés d'invités d'honneur 23 communications par affichage. 330 personnes ont assisté au atelier.

Le 18e atelier annuel sur la toxicité aquatique a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un Comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS/REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commerical products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur; ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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TABLE OF CONTENTS/TABLE DES MATIÈRES

Page

PREFACE/PREFACE iii

EDITORS COMMENTS/REMARQUES DES EDITEURS iv

ORGANIZING COMMITTEE/COMITÉ D'ORGANISATION vi

WORKSHOP SPONSORS/COMMANDITAIRES DES ATELIERS vii

TABLE OF CONTENTS/TABLE DES MATIÈRES v

PLENARY SESSION/SÉANCE PLÉNIÈRE 1

REGULATORY USES OF AQUATIC TOXICOLOGY (PITFALLS AND OPPORTUNITIES). P.M. Chapman. 2

APPLICATION OF AQUATIC TOXICOLOGY IN ENVIRONMENT CANADA'S REGULATION PROGRAM. G. Allard. 13

BIOAVAILABILITY OF METALS IN THE AQUATIC ENVIRONMENT: ANALYTICAL, GEOCHEMICAL AND BIOLOGICAL ASPECTS. P.G.C. Campbell. 13

SESSION 1A: ABIOTIC FACTORS WHICH INFLUENCE CONTAMINANT TRANSFER/FACTEURS ABIOTIQUE QUI INFLUENCENT LE TRANSFERT DES CONTAMINANTS 14

CHARACTERISTICS OF COHESIVE SEDIMENTS DURING SETTLING. Y.L. Lau. 15

TRANSPORT OF PCBs IN THE OTONABEE RIVER-RICE LAKE SYSTEM. C.D. Metcalfe, B.G. Koenig, M.L. Ferguson and C.R. Macdonald. 22

BIOAVAILABILITY OF TRACE ELEMENTS ASSOCIATED WITH PARTICULATES. S.N. Luoma, N.S. Fisher, J. Reinfelder and A.W. Decho. 26

ROLE DE LA CHIMIE DU SOUFRE SUR LA REMOBILISATION DU MERCURE DANS LES SÉDIMENTS DU FJORD DU SANUENAY. C. Gagnon, E. Pelletier et A. Mucci. 27

SEDIMENT/WATER DISTRIBUTION COEFFICIENTS AND SPECIATION OF CADMIUM IN FRESH WATERS. R. Wagemann, M. Capel and R. Hesslein. 35

SOLUBILITY ENHANCEMENT OF FENVALERATE BY ISOLATED DOC LAKEWATER FRACTIONS. B.K. Burnison. 36

RESUSPENSION OF PARTICLE BOUND CONTAMINANTS IN THE GREAT LAKES. M.N. Charlton and M.E. Fox. 36

COMPLEXATION OF RADIONUCLIDES AND TRACE METALS BY DISSOLVED ORGANIC CARBON. R.J. Cornett, L.A. Chant and C.M. Milton. 37

ABIOTIC FACTORS THAT INFLUENCE TRACE METAL DISTRIBUTION AMONG SEDIMENT AND SOLUTION PHASES IN A SMALL URBANIZED RIVER. L.A. Warren 37 and A.P. Zimmerman.

SESSION 1B: BIOTIC FACTORS WHICH INFLUENCE CONTAMINANT TRANSFER/FACTEURS BIOTIQUES QUI INFLUENCENT LE TRANSFERT DES CONTAMINANTS 38

BIOCONCENTRATION ET DISTRIBUTIONÀ TRÈS COURT TERME DE $^{203}HgCl_2$ ET $CH_3{}^{203}HgCl$ CHEZ L'ÉTOILE DE MER Asterias rubens. C. Rouleau, E. Pelletier et H. Tjälve. 39

FATE OF MERCURY AND METHYLMERCURY IN STARFISH Leptasterias polaris: ORGAN DISTRIBUTION, FLUXES AND TRANSPORT MECHANISMS. S. Maheu et E. Pelletier. 49

RELATIONSHIPS BETWEEN Cd^{2+} ACTIVITY CADMIUM BIOACCUMULATION AND SUBLETHAL TOXICITY IN THE POLYCHAETE Neanthes arenaceodentata. K.J. Jenkins and A.Z. Mason. 61

ADJUSTING CONTAMINANT CONCENTRATIONS FOR FISH-SIZE COVARIATION. K.M. Somers and D.A. Jackson. 63

WHAT LIMITS THE RATE OF METHYLMERCURY UPTAKE VIA THE GILLS OF FISH? G. Mierle. 65

BIOACCUMULATION DE MÉTAUX LOURDS CHEZ UN AMPHIPODE DU FLEUVE SAINT-LAURENT EN RELATION AVEC LA CONTAMINATION DES SÉDIMENTS. M. Amyot, B. Pinel-Alloul et P.G.C. Campbell. 67

PROBLEMS IN THE USE OF BIOLOGICAL INDICATORS OF METAL CONTAMINATION. W.J. Langston. 81

VARIATION OF Cd BODY CONCENTRATIONS AND BURDENS IN AQUATIC INVERTEBRATES. D.C. Lasenby, R.D. Evans and N.D. Yan. 81

DEGRADATION DU CHLORURE DE TRIBUTYLETAIN PAR L'ALGUE MARINE Pavlova lutheri; EFFETS SUR LA CROISSANCE DE L'ALGUE. R. St-Louis, E. Pelletier, P. Marsot et R. Fournier. 82

SESSION 1C: WHOLE ECOSYSTEM ASPECTS OF CONTAMINANT TRANSFER/ASPECTS ÉCOSYSÉMIQUES GLOBAUX DU TRANSFERT DES CONTAMINANTS 83

AQUATIC TOXICOLOGY - THE WIDER IMPLICATIONS OF EASTERN CANADIAN SHELLFISH TOXINS. D.J. Scarratt. 84

RELATIVE IMPORTANCE OF MACROPHYTE SPECIES AS CADMIUM SINKS IN A SHIELD LAKE RECEIVING EXPERIMENTAL ADDITIONS OF CADMIUM. D.F. Malley, M. Shaw, M. Thibodeau and D. Huebert. 86

A REGIONAL ASSESSMENT OF MERCURY CONTAMINATION IN YELLOW PERCH (Perca flavescens) IN ONTARIO: IMPLICATIONS FOR COMMON LOONS (Gavia immer). I.D. Cuthbert. 87

DETERMINANTS OF THE SHORT-TERM DYNAMICS OF PCB UPTAKE BY PLANKTON. G. Richer and R.H. Peters. 98

THE ROLE OF SEDIMENT AND FOOD WEB STRUCTURE IN THE DISTRIBUTION OF PCB CONGENERS IN SMALL LAKES. C.R. Macdonald and C.D. Metcalfe. 101

WHY DON'T GREAT LAKES FISH REFLECT ENVIRONMENTAL LEVELS OF ORGANIC CONTAMINANTS. D.J. Rowan and J.B. Rasmussen. 101

BIOACCUMULATION OF PCBs IN ONTARIO FISH. E. Bentzen, D.R.S. Lean, C. Hauschield and W.A. Scheider. 102

LIPID CONTAMINANT INTERACTIONS. B.C. Wainman and D.R.S. Lean. 102

SESSION 2A: APPROACHES TO EVALUATING THE ECOLOGICAL RELEVANCE OF BIOMARKERS OF STRESS/MÉTHODES D'ÉVALUATION DE LA PERTINENCE, SUR LE PLAN ÉCOLOGIQUE, DES BIOINDICATEURS DES MARQUEURS BIOLOGIQUES DE STRESS 103

BEHAVIORAL BIOMARKERS INDICATIVE OF STRESS IN *Daphnia*. D.C. McNaught and D.C. Drake. 104

SIGNIFICANCE OF LIVER NEOPLASIA IN WILD FISH: ASSESSMENT OF PATHOPHYSIOLOGIC RESPONSES OF A BIOMONITOR SPECIES TO MULTIPLE STRESS FACTORS. G.M. Kirby and M.A. Hayes. 106

DOES EXPOSURE TO GENOTOXINS INFLUENCE GENETIC DIVERSITY PATTERNS IN NATURAL FISH POPULATIONS? M.H. Murdoch and P.D.N. Hebert. 117

CARACTÉRISATION DE LA RÉPONSE D'UN INDICATEUR CYTOCHIMIQUE DE POLLUTION FACE AUX FACTEURS ENVIRONNEMENTAUX NATURELS. R. Tramblay et J. Pellerin. 120

AN INTERLABORATORY COMPARISON OF THE ETHOXYRESORUFIN-O-DEETHYLASE ASSAY FOR MFO ACTIVITY. K.R. Munkittrick, M.R. van den Heuvel, J.J. Stegman, D.A. Metner, W.L. Lockhart, J.F. Paine, P.V. Hodson, S. Kennedy, J. Bureau, I.R. Smith, M. Adams, J.A. Miller and P. Martel. 123

HISTOPATHOLOGIE DES BRANCHIES ET DU FOIE DE POISSON COMME INDICATEUR DE LA QUALITÉ DES HABITATS AQUATIQUES DU SAINT-LAURENT. B. Morin, G. Walsh, C. Audet et L. Lapierre. 128

REDUCTION IN PAH CAUSES DECLINE IN LIVER NEOPLASMS IN BLACK RIVER BILLHEAD. P.C. Baumann. 131

BIOMARKERS: ACADEMICALLY INTERESTING OR USEFUL INDICATORS. J.H. McCormick. 132

SESSION 2B: MESOCOSMS IN THE STUDY OF FATE AND EFFECTS OF TOXIC CHEMICALS: HOW FAR HAVE WE ADVANCED?/LES MÉSOCOSMES DANS L'ÉTUDE DU DEVENIR ET DES EFFETS DES PRODUITS CHIMIQUES TOXIQUES: OÙ EN SOMMES NOUS RENDUS? 133

EFFETS D'UN DÉVERSEMENT DE PÉTROLE EXPÉRIMENTAL SUR LA FLORE BACTÉRIENNE DE L'ESTUAIRE DU SAINT-LAURENT. R. Simon, E. Pelletier et D. Delille. 134

THE ACCURACY AND PRECISION OF PESTICIDE CONCENTRATIONS FOLLOWING APPLICATION TO LITTORAL ENCLOSURES. M.L. Knuth. 137

HERBICIDE CONCENTRATIONS IN THE WATER AND SURFACE FILM OF SOME SASKATCHEWAN PONDS. D.T. Waite, R. Grover, L. Kerr and R. Hopkinson. 138

BIOAVAILABILITY OF SEDIMENT ASSOCIATED DIOXIN CONGENERS TO MUSSEL AND CRAYFISH IN FRESHWATER ENCLOSURES. M.D. Segstro, M.R. Servos, G.R.B. Webster and D.C.G. Muir. 139

MICROBIAL GENE TRANSFER IN RESPONSE TO CHLOROAROMATIC CHEMICAL EXPOSURE; FRESHWATER MESOCOSMS AND INDUSTRIAL MICROCOSMS. R.C. Wyndham, A. Cashore, R. Fulthorpe, C. Nakatsu, J. Ng, M. Peel and F. Szilagyi. 139

SESSION 2C: METHODS TO DETERMINE FATE AND EFFECTS OF CONTAMINANTS IN LOTIC ENVIRONMENTS/MÉTHODES VISANT À DÉTERMINER LE DEVENIR ET LES EFFETS DES CONTAMINANTS EN MILIEU LOTIQUE 140

GROWTH RATES OF UNIONID BIVALVES UPSTREAM AND DOWNSTREAM OF INDUSTRIAL OUTFALLS CONTAMINATED WITH TRACE METALS. L.C. Grapentine. 141

THE EFFECT OF EDTA ON CADMIUM AND ZINC UPTAKE AND TOXICITY IN THE SUBMERGED AQUATIC MACROPHYTE *Lemma trisulca* L. D.B. Huebert and J.M. Shay. 144

PCB DYNAMICS IN A SOUTHWESTERN ONTARIO CREEK. D.T. Zaranko, N. Kaushik, K. Solomon and R.W. Griffiths. 148

QUANTIFYING CONTAMINANT DYNAMICS IN THE HURON - ERIE CORRIDOR. G.D. Haffner and F.A.P.C. Gobas. 161

UTILISATION DE LA STERNE PIERREGARIN COMME SONDE BIOANALYTIQUE DES NIVEAUX DE CONTAMINATION PAR LES ORGANOCHLORÉS ET LES CHLOROPHÉNOLS DANS LE SYSTEME DU SAINT-LAURENT. E. Razurel, E. Pelletier et J.L. Desgranges. 161

SESSION 3A: BIOLOGICAL TESTING OF EFFLUENTS AND RECEIVING WATER TO PREDICT ENVIRONMENTAL IMPACTS/TESTS BIOLOGIQUES SUR LES EFFLUENTS ET LES EAUX RÉCEPTRICES AFIN DE PRÉDIRE LES RÉPERCUSSIONS ÉCOLOGIQUES 162

BIOLOGICAL TESTING: A KEY COMPONENT OF AQUATIC ENVIRONMENTAL EFFECTS MONITORING AT PULP AND PAPER MILLS. R.P. Scroggins and W.R. Parker. 163

THE ACUTE LETHALITY OF SAMPLES OF LIQUID EFFLUENT FROM PULP AND PAPER MILLS IN ONTARIO TO RAINBOW TROUT AND TO *Daphnia magna*. S.G. Abernethy. 166

THE USE OF AQUATIC PLANT TOXICITY TESTS IN BIOMONITORING PROGRAMS. J.S. Hughes. 169

EVALUATION OF THE TOXICOLOGICAL EFFECTS OF OIL DISPERSANTS BY MODELED-EXPOSURE TOXICITY TESTING. M.M. Singer, R.S. Tjeedema and D.L. Smalheer. 175

DIAGNOSIS OF EFFLUENT ECOTOXICITY WITH AN INNOVATIVE BIOLOGICAL/CHEMICAL APPROACH. G. Costan. 183

COMPARISON OF CHEMICAL CHARACTERISTICS OF EFFLUENTS FROM 3 CANADIAN KRAFT PULP MILLS. J.H. Carey, D.T. Bennie and B.K. Burnison. 183

DEVELOPPEMENT D'UN TEST DE LETALITE ALGALE PAR CYTOMETRIE EN FLUX. L. Ménard, C. Blaise et P. Couture. 184

INFLUENCE DU L'EAU DE DILUTION SUR LES RESULTATS DE BIOESSAIS EFFECTUES SUR DES ECHANTILLONS D'EFFLUENTS INDUSTRIELS. D. St-Laurent et C. Blaise. 184

INTEGRATION OF PYHSICAL, CHEMICAL AND BIOLOGICAL DISCHARGE EFFECTS IN RECEIVING ENVIRONMENTS. G.R. Craig. 185

TOWARDS A MORE COMPREHENSIVE USE OF BIOANALYTICAL TOOLS FOR INDUSTRIAL EFFLUENT CONTROL. N. Bermingham, G. Costan and Y. Roy. 185

SESSION 3B: ENVIRONMENTAL EFFECTS MONITORING USING INDIGENOUS BIOTA IN RECEIVING WATERS/SURVEILLANCE DES RÉPERCUSSIONS ENVIRONMENTALES À L'AIDE DE BIOTE INDIGÈNE DANS LES EAUX RÉCEPTRICES 186

TENNESSEE'S EAST FORK POPLAR CREEK: A BIOLOGICAL MONITORING AND ABATEMENT PROGRAM. R.S. Halbrook, J.M. Loar, S.M. Adams, M.C. Black, H.L. Boston, A.J. Gatz, M.S. Greeley, Jr., W.R. Hill, R.L. Hinzman, J.F. McCarthy, M.J. Peterson, M.G. Ryon, E.M. Schmilling, J.G. Smith, G.R. Southworth and A.J. Stewart. 187

MFO MEASUREMENTS IN ENVIRONMENTAL REGULATION. R.F. Addison. 189

COMPARISON OF WILD FISH POPULATIONS BEFORE AND AFTER SECONDARY TREATMENT OF BLEACHED KRAFT MILL EFFLUENT. K.R. Munkittrick, M.E. McMaster, C.B. Portt, G.J. Van Der Kraak and I.R. Smith. 190

EVALUATION OF BIOINDICATORS OF CONTAMINANT-EXPOSURE AND EFFECTS IN COASTAL ECOSYSTEMS. U. Varanasi, J.E. Stein, T.K. Collier, L.L. Johnson, E. Casillas and M.S. Myers. 198

SESSION 3C: BIOLOGICAL APPROACHES TO SITE-SPECIFIC EFFLUENT REGULATION/APPROCHES BIOLOGIQUES À LA REGLEMENTATION DES EFFLUENTS À DES ENDROITS DONNÉS 199

LES CRITÉRES DE QUALITÉ DE L'EAU AU QUÉBEC: DÉFINITIONS ET APPLICATION. I. Guay. 200

ONTARIO'S PROVINCIAL WATER QUALITY OBJECTIVES AND THEIR USE IN SETTING SITE-SPECIFIC WATER QUALITY CRITERIA. D.J. Spry. 214

INTERNATIONAL APPROACHES TO ENVIRONMENTAL EFFECTS MONITORING. K. Clarke-Whistler and J. Miller. 218

ENVIRONMENT CANADA'S INITIATIVE ON ENVIRONMENTAL EFFECTS MONITORING: TECHNICAL DEVELOPMENT. G.R. Craig, P. Orr, D. Hart and R. Scroggins. 219

ALBERTA ENVIRONMENT'S USE OF ECOLOGICAL MONITORING IN EFFLUENT LICENSING. J.B. Kemper, D.O. Trew, L.R. Noton and I. Mackenzie. 219

RECENT DEVELOPMENTS IN THE U.S.A. ON THE WATER QUALITY BASED APPROACH AND ECOSYSTEM MONITORING. R. Brandes. 220

SESSION 3D: AQUATIC TOXICOLOGY IN SUPPORT OF THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA)/LA TOXICOLOGIE AQUATIQUE À L'APPUI DE LA LOI CANADIENNE SUR LA PROTECTION DE L'ENVIRONNEMENT (LCPE) 221

DEVELOPMENT OF 10-DAY MARINE/ESTUARINE AMPHIPOD ASSAY FOR SEDIMENT TOXICITY IN SUPPORT OF THE OCEAN DUMPING PROGRAM (CEPA, PART VI). D.J. McLeay, S.G. Yee, K.G. Doe and L.M. Porebski. 222

EVALUATION OF FOUR FRESHWATER BENTHIC INVERTEBRATES FOR ASSESSMENT OF CONTAMINATED SEDIMENTS AND THEIR RELEVANCE TO CEPA. K.E. Day, and T.B. Reynoldson. 235

ASSESSMENT OF NEW CHEMICALS FOR THE CANADIAN ENVIRONMENTAL PROTECTION ACT. R.A.F. Matheson. 238

TOXICITY ASSESSMENT OF BIOTECHNOLOGY PRODUCTS UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA). T. Edge and T. McIntyre. 238

INTERNATIONAL HARMONIZATION OF ENVIRONMENTAL TESTING PROCEDURES. G.R. Biddinger, S.J. Pauwels and M.L. Hinman. 239

OCEAN DUMPING: SEDIMENT QUALITY GUIDELINES IN A REGULATORY FRAMEWORK/IMMERSION DE DÉCHETS EN MER: LES RECOMMANDATIONS SUR LA QUALITÉ DES SÉDIMENTS À L'INTÉRIEUR D'UN CADRE LÉGISLATIF 240

A PROTOCOL FOR THE DERIVATION AND USE OF CANADIAN SEDIMENT QUALITY GUIDELINES. D.D. MacDonald and S.L. Smith. 241

EVALUATION OF DREDGED MATERIAL FOR OPEN WATER DISPOSAL: THE U.S. ARMY CORPS OF ENGINEERS' EFFECTS-BASED TESTING PROGRAM. T. Dillon. 242

PROVINCIAL SEDIMENT QUALITY GUIDELINES. R. Jaagumagi. 242

BIOLOGICAL SEDIMENT CRITERIA. T.B. Reynoldson and K.E. Day. 243

ENVIRONMENT CANADA'S OCEAN DUMPING CONTROL PROGRAM. L. Porebski and J. Karau. 243

SPECIAL INTERNATIONAL SESSION ON CONTAMINANTS IN THE ARCTIC/SÉANCE SPÉCIALE INTERNATIONALE SUR LES CONTAMINANTS DANS L'ARCTIQUE 244

TEMPERATURE AND THE GLOBAL DISTRIBUTION OF LOW VOLATILE ORGANIC COMPOUNDS. F. Wania and D. Mackay. 245

SELECTIVE ACCUMULATION OF POLYCHLORINATED CAMPHENES IN AQUATIC BIOTA FROM THE CANADIAN ARCTIC. T.F. Bidleman, M.D. Walla and D.C.G. Muir. 253

PCB METHYLSULPHONES IN MAMMALS FROM THE CANADIAN AND SWEDISH ENVIRONMENTS. Å. Bergman, H. Kuroki, K. Haraguchi and R.J. Norstrom. 259

POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS FROM ARCTIC AND MIXED-FUNCTION OXIDASE ENZYMES IN FISH FROM THE SAME LAKES. W.L. Lockhart, B.N. Billeck, D.A. Metner and G.J. Brunskill. 264

BREAST MILK CONTAMINATION BY PCDDs, PCDFs, PCBs, COPLANAR PCBs AND CHLORINATED PESTICIDES IN ARCTIC QUÉBEC. E. Dewailly, A. Nantel, S. Bruneau, C. Laliberté, J.P. Weber and S. Gingras. 265

ORGANOCHLORINES AND HEAVY METALS IN THE BERING/CHUKCHI SEA ECOSYSTEMS. C.P. Rice, S.M. Chernyak, D. Hinckley, A. Krynitsky and T. Kolobova. 269

CHROMIUM (III) CONCENTRATIONS IN THE RECIPIENT OUTSIDE A TANNERY IN SOUTH GREENLAND. C.M. Glahder. 272

HEAVY METALS AND SELENIUM IN ARCTIC MARINE MAMMALS. R. Wagemann and R. Stewart. 278

RECENT STUDIES ON HEAVY METALS IN POLAR BEARS FROM GREENLAND WITH REFERENCE TO OTHER MARINE MAMMALS. R. Dietz and C.T. Agger. 280

MARINE MAMMAL TISSUE ARCHIVE PROGRAM. P.R. Becker and S.A. Wise. 287

U.S. EPA'S ARCTIC CONTAMINANT RESEARCH PROGRAM: STRATEGY AND APPROACH. D.H. Landers, J. Ford and C.P. Gubala. 292

CONTAMINANTS IN ARCTIC ALASKA: LICHEN AND MOSS STUDIES. J. Ford, R. Thomas and D. Landers. 296

CONTAMINANTS IN NORTHERN QUÉBEC: LEVELS AND TRENDS. H. Careau, A. Vézina, D. Gauvin and E. Dewailly. 299

SOURCES AND PATHWAYS OF ARCTIC CONTAMINANTS. L.A. Barrie. 305

DEPOSITION AND FATE OF SEMI-VOLATILE TRACE ORGANICS IN ARCTIC SNOWPACKS. D.J. Gregor. 308

MONITORING OF CONTAMINANT LEVELS IN THE ENVIRONMENTAL 308

COMPONENTS OF THE SIBERIAN SHELF SEAS. RESULTS OF FIVE YEAR STUDIES. S. Melnikov.

PATTERNS AND TRENDS OF CONTAMINANTS IN THE ECOSYSTEMS OF THE CHUKCHI AND BERING SEAS. S.M. Chernyak. 309

BIOGEOCHEMICAL STUDIES OF PCB IN ARCTIC ECOSYSTEMS. A.V.Tysban, S.M. Chernyak and G.V. Paniv. 309

ACCUMULATION OF CHLORINATED HYDROCARBONS AND HEAVY METALS IN HIGHER TROPHIC LEVEL ORGANISMS OF THE BARENTS SEA ECOSYSTEM. T.N. Savinova. 310

ORGANOCHLORINE LEVELS IN ARCTIC RINGED SEALS, 1972 - 1989. R.F. Addison. 310

CIRCUMPOLAR SURVEY OF ORGANOCHLORINES IN POLAR BEARS: PRELIMINARY RESULTS. R.J. Norstrom. 311

THE U.S. ARCTIC CONTAMINANT RESEARCH PROGRAM: CHEMICAL SEDIMENT STRATIGRAPHIES OF TWO ALASKAN LAKES. C.P. Gubala, D.H. Landers, M. Monetti and R. Thomas. 311

ACCUMULATION OF COPLANAR PCBs IN ARCTIC MARINE MAMMALS AND FISH. D.C.G. Muir, C.A. Ford, M.D. Segstro, R.J. Norstrom and M. Simon. 312

LEVELS OF PCDD/PCDF AND COPLANAR PCB IN BIOTA AND SEDIMENT FROM THE NORWEGIAN ARCTIC. M. Oehme, M. Schlabach and A. Biseth. 312

POSTERS/L'AFFICHE SCIENTIFIQUE 313

TOXIC EQUIVALENT FACTORS (TEFS) AND TISSUE DISTRIBUTION FOR SEVERAL 2,3,7,8-SUBSTITUTED DIOXINS IN RAINBOW TROUT. J.L. Parrott, P.V. Hodson, D.G. Dixon and M.R. Servos. 314

ACID VOLATILE SULFIDE (AVS) IN A SEASONALLY ANOXIC MESOTROPHIC LAKE: SEASONAL AND SPATIAL CHANGES IN SEDIMENT AVS. D.E. Howard and R.D. Evans. 317

RÉPONSES BIOCHIMIQUES AU CADMIUM CHEZ *Anodonta grandis* EN MILIEU AQUATIQUE. J. Pellerin-Massicotte, C. St-Pierre, E. Mayrand, P. Campbell, A. Tessier et Y. Couillard. 325

BIOCONCENTRATION ET DISTRIBUTION DE $^{54}Mn^{2+}$ ET EFFETS D'AGENTS COMPLEXANTS CHEZ LA TRUITE BRUNE *Salmo trutta*. C. Rouleau, E. Pelletier et H. Tjälve. 330

ANALYSIS OF TRIALKYLTIN IN ESTUARINE SEDIMENTS AND OYSTER TISSUES. R.G. McPherson and K.R. Brown. 340

STUDY OF COPPER AND ZINC LEVELS IN ESTUARIES USING THE SYDNEY ROCK OYSTER AS A BIOMONITOR. K.R. Brown and R.G. McPherson. 345

LE TRANSFERT DES BIPHÉNYLS POLYCHLORÉS DES SÉDIMENTS AUX 349

GASTÉROPODES HERBIVORES VIA LE PÉRIPHYTON. C. Vanier et D. Planas.

EMERGING INSECTS AS A BIOTIC PATHWAY FOR MOVEMENT OF 2,3,7,8-TETRACHLORODIBENZOFURAN FROM LAKE SEDIMENTS. W.L. Fairchild, D.C.G. Muir, R.S. Currie and A.L. Yarechewski. 350

WHY ARE ST. LAWRENCE BELUGA WHALES SO CONTAMINATED? A PHARMACOKINETICS ANALYSIS. B.E. Hickie, P.V. Hodson, P. Béland and D. Mackay. 350

CONTAMINATION LEVELS OF RECENT SEDIMENTS SAMPLED IN THE UPPER SAGUENAY FJORD (QUÉBEC). E. Pelletier, B. Sainte-Marie and P. Hodson. 351

FATE OF ORGANOCHLORINES DOWNSTREAM OF AN ALBERTA BLEACHED KRAFT PULP MILL. S.M. Swanson and D.A. Birkholz. 351

HOW DO WE HANDLE THE REGISTRATION OF COPPER-BASED PRODUCTS? P.-Y. Caux. 352

CAN CHRONIC CYANIDE EXPOSURE INDUCE CHRONIC THIOCYANATE TOXICITY IN RAINBOW TROUT? R.P. Lanno and D.G. Dixon. 352

INFLUENCE OF EXPOSURE TIME ON TISSUE DISTRIBUTION OF MERCURY IN RAINBOW TROUT. A.J. Niimi and G.P. Kissoon. 353

COPPER AND CADMIUM BINDING TO FISH GILLS: EFFECTS OF COMPETITION AND COMPLEXATION. R.C. Playle and D.G. Dixon. 353

CADMIUM AND LEAD CONCENTRATIONS OF *Nuphar variegatum* IN RELATION TO WETLAND TYPE. E. Thompson, F. Pick and L.I. Bendell-Young. 354

TRACE METALS IN SEDIMENTS INFLUENCED BY COPPER MINE TAILINGS. R.G. Trucco, I. Inda and M.L. Fernández. 354

EVALUATION DE LA TOXICITE ENVIRONNEMENTALE PAR DES INDICES BIOCHIMIQUES DE CROISSANCE. E. Mayrand et J. Pellerin. 355

HEPATIC BIOCHEMICAL OBJECTIVES TO PROVIDE SAFE LEVELS FOR RAINBOW TROUT. C.J.P. McKean and J. Deniseger. 355

ON SITE TREATMENT OF ST. MARYS RIVER SEDIMENTS. T. Murphy, K. McCabe and M. Fox. 356

COAL TAR CONTAMINATION OF HAMILTON HARBOUR. T. Murphy, A. Moller, M. Fox, E. Nagy and M.S. Burgham. 356

THE ROLE OF SEDIMENT pH AND REDOX POTENTIAL IN DETERMINING METAL BIOAVAILABILITY TO ISOETID MACROPHYTES. L.J. Jackson, J. Kalff and J.B. Rasmussen. 357

CAN PLANT TOXIC COMPOUNDS AFFECT AQUATIC ORGANISMS? A.M. Zobel and C. Santos. 357

BEST STUDENT AWARD/PRIX POUR LE MEILLEUR ÉTUDIANT 358

LIST OF AUTHORS/LISTE DES AUTEURS 359

LIST OF REGISTRANTS/LISTE DES PARTICIPANTS 361

ADDENDUM/ADDENDA: 16th Annual Aquatic Toxicity Workshop, November 6-8, 1989, Winnipeg, Manitoba. 368

LONG-TERM EFFECTS OF AN ACUTE ACID PULSE ON JUVENILE BLACK CRAPPIE, Pomoxis nigromaculatus. R.L. Leino, J.H. McCormick and K.M. Jensen. 369

THE EFFECTS OF BOREAL, SUBARCTIC VERY-SOFT WATERS AND URANIUM EFFLUENTS ON SOME POPULATION CHARACTERISTICS OF Ceriodaphnia dubia (CRUSTACEA: CLADOCERA). G.E. Melville. 372

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER 380

PLENARY SESSION/SÉANCE PLÉNIÈRE

CHAIRPERSON/PRÉSIDENTE

Janet Davies

REGULATORY USES OF AQUATIC TOXICOLOGY (PITFALLS AND OPPORTUNITIES). P.M. Chapman, E.V.S. Consultants Limited, North Vancouver, British Columbia.

ABSTRACT

Toxicity tests, used with common sense, provide one of the best available means to determine the potential environmental hazard of past, present and future (=proposed) practices. Such usage typically involves burden of evidence, most sensitive species, worst case exposures and most sensitive measures. Potential does not, of course, necessarily equate with reality. However, common sense is generally absent from the highly legalistic regulatory arena in North America where environmental legislation has been, and is almost certain to remain, highly proscriptive. As a result, end-points in toxicity tests become the end in themselves rather than the means to "preserve and protect". The current trend to developing more and more sensitive tests, whose end-points are not necessarily related to environmental significance, is not useful, needs to be tempered with common sense (=best professional judgement based on burden of evidence), and ideally should evolve to the point where we can avoid surrogates and directly test what we want to protect. Otherwise, the usefulness of toxicity tests and their general acceptance may be lost. Most importantly, we will not be achieving our ultimate goal of environmental protection.

*Plenary presentation at the 18th Annual Aquatic Toxicity Workshop, September 30 - October 03, 1991, Ottawa, Ontario.

INTRODUCTION

Bioassays, in the form of toxicity testing, provide a sharp-edged tool for environmental assessment and regulation. Unfortunately, much like the legendary Sword of Damocles, this tool is two-edged (and equally sharp on each side). It can be both used (opportunities) and misused, ultimately rebounding on the user (pitfalls). In this paper, I provide a personal view of some of the opportunities and pitfalls inherent in regulatory uses of aquatic toxicology. For purposes of readability (and to increase the probability that these words will actually be read), I have tried to keep my comments short and to the point. Readers who wish to discuss and/or argue this subject at greater length are encouraged to contact me directly (phone: 604-986-4331; fax 604-662-8548).

PITFALLS AND OPPORTUNITIES

There are two philosophical bases for environmental regulation:

- homocentric (=for human beings primarily)
- biocentric (=for Gaia, i.e., the Earth and its ecology as a whole; human beings are secondary at best).

While the latter concept holds the greatest appeal to those who consider themselves most dedicated to and concerned with the environment, I do not believe that we can adequately protect the environment from ourselves by appealing to abstract philosophical principles. We can only protect the environment by convincing the vast majority of human beings, in both developed and developing countries, that environmental protection is in their best [selfish and self-centered] interests. To do this, we must avoid "crying wolf"; we must be sure we are dealing with real problems; and, we must address the most important problems first and foremost. It is within the context of this pragmatic but, I believe, eminently workable philosophy, that I discuss the following pitfalls and opportunities of regulatory aquatic toxicity testing.

Opportunity/Pitfall #1

Opportunity: Ability to be proactive

In the past, environmental actions have generally been reactive (i.e., there is a problem now) rather than proactive (i.e., a prediction that there will be a problem in future if certain actions are not taken). By using toxicity tests under "worst case" scenarios (i.e., most sensitive species, most sensitive life-stages, most severe laboratory exposure scenarios, likely most toxic and contaminated test samples), we have the use of an excellent tool to predict whether a problem may occur before it is manifest in the environment.

Pitfall: Assumption that "worst case" scenario will occur

The pitfall to this opportunity is the necessarily conservative outlook of regulators, who tend to assume that "worst case" will occur, or that even worse things may happen. This outlook does not lend itself to realistic appraisals and actions. "Worst case", like Christmas Future in the story, A Christmas Carol, need not come to pass if we act appropriately. For instance (Chapman et al., 1991), despite elevated sediment chemical contamination and toxicity in the immediate vicinity of an oil platform in the Gulf of Mexico, effects were not manifest in the resident benthic communities. This lack of effects indicated caution in imposing any additional stresses on the system and suggested that present stresses be reduced. It did not indicate a need to "clean up" a non-problem.

Opportunity/Pitfall #2

Opportunity: Effects-based regulation and decision-making

A clear opportunity exists to use effects based toxicity testing to answer the "So what?" question, and to do so under predictive, "worst case" conditions. Clearly, if a chemical does not cause an effect, it is not of concern, whereas the level of concern (and hence of prioritization for control, remedial action, etc.) increases exponentially as the level(s) of effect(s) increase.

Pitfall: Failure to update regulation and decision-making

Toxicity testing, although dating back to Aristotle (who observed sludge worms downstream of inputs from Athens and then tested the reactions of these worms to salt and fresh water), has only relatively recently assumed primary importance in the regulatory arena (e.g., the Canadian Environmental Protection Act, CEPA). Toxicity testing, in many cases, replaces previous measures of bioavailability derived from expensive body-residue analyses except where food chain transfers are of concern. For some chemicals of concern (e.g., PCBs), for which we do not have appropriate toxicity tests, and for the few chemicals (i.e., PCBs, mercury, DDT, and some dioxins) which clearly biomagnify (=bioconcentrate up the food chain), tissue residue analysis is still necessary. Otherwise, such analyses should be replaced by appropriate toxicity tests. However, note that biomagnification has only been shown to occur in mammals (e.g., mink, seals) and in birds (e.g., heron, eagles). There is no evidence that biomagnification occurs in aquatic food chains (e.g., fish)(SAB, in press).

Opportunity/Pitfall #3

Opportunity: Use of appropriate end-points

I have argued elsewhere (Chapman, 1991) that, at the organism level, three measures are required to provide information concerning the stability of populations and of higher levels of organization: survival, growth and reproduction. If an organism can fulfill all of these integrated energetic functions, then it is not being adversely affected. In an ideal world, we would test the tolerance and response of a wide range of individuals, life-stages, species, taxa, populations, communities and ecosystems. And we would do so to all possible perturbations, individually and in mixtures. Clearly this is not possible in the real world, where there is a high level of uncertainty precisely because we cannot (and should not try to) measure everything. However, this uncertainty can be reduced by:

- identifying and concentrating on key taxa and processes
- wherever possible, making direct measurements and avoiding surrogates.

If we conduct tests on key taxa, in particular those which we want to protect, and we avoid surrogates, then we have the best, least ambiguous information for environmental regulation. Key taxa are those that perform unique individual actions which have an important role in ecosystem function and stability.

Pitfall: Lack of clarity - end-points and their role

End-points are all too often perceived as being the end rather than the means. We measure end-points such as mortality to determine whether what we are testing can kill individuals and often do so with surrogates (e.g., daphnids exposed to sediment elutriates); if death occurs, we then need to determine what this means in the real environment outside the laboratory, where many of the surrogates we test would not be naturally found in close proximity to what we are testing. Too often regulators incorrectly use surrogate end-points as absolute and complete measures. Moreover, we are often not clear in describing our test end-points (what exactly is a "sublethal chronic" test?). I have previously (Chapman, 1989a) pointed out the need to be precise in describing test end-points, most especially since regulations are written by and for lawyers.

Opportunity/Pitfall #4

Opportunity: Test what is important and relevant

If we test the appropriate end-points on the appropriate organisms, we have the best possible information for regulation. Choice of organisms should include (cf. Opportunity/Pitfall #3, above), whenever possible, key taxa which we choose to protect, rather than surrogates. Determining the beneficial uses of a system which are at risk and any possible effects from expected stresses, provides guidance in determining what to test, how and for what. In this manner, we can focus our efforts on "worst case" scenarios and determine whether (or not) these might be realized.

Pitfall: Don't lose perspective

There are several pitfalls to this opportunity, including our very human desire to use our favorite test(s), sometimes despite the fact that these are not useful. Sometimes, also, we like to use "trendy" tests (check the titles of papers at a conference; the test most referred to is the latest "trendy" test) which, again, may or may not apply. Another pitfall is the common failure to maintain a point of reference before, during and after testing (What is the study question? Why does it matter?). A final pitfall is, as discussed previously (cf. Opportunity/Pitfall #3), a fatal desire to try to test everything, every way.

Opportunity/Pitfall #5

Opportunity: If no problem exists, stop

If appropriate tests, under "worst case" conditions, indicate no cause for concern, these are the results. There are many major problems requiring our attention; the more we can focus our efforts on major, real problems, the better. A toxicity test comprises the answer (end-point/effect) to a question (stress/insult) posed to a biological system. For the sake of the environment, if nothing else, "let the critters tell it like it is!" Conversely, if appropriate tests, under "worst case" conditions, indicate a potential or real cause for concern, there are three possible responses:

1) if clearly required, control/regulate

2) if results are unclear, test/verify further

3) if clearly there is no problem, stop (do not regulate or test further).

To determine the appropriate response, you may need to check what other indicators (chemical measurements, available information on resident organisms) tell you; there may or may not be a real problem. Find out. If burden of evidence and best professional judgment indicates that the problem is real, address it; if it is not, move onto more pressing issues.

Pitfall: The "bottomless pit of increasingly meaningless tests"

These is a distressingly lunatic modern tendency among some members of the regulatory community to pose an endless series of "what if" questions which are open-ended and basically unanswerable but amenable to endless study. We will never totally understand biological systems, but we do know enough now to differentiate immediate, major problems (e.g., massive mortalities in Eastern European water bodies) from inconsequential or minor problems (e.g., deaths in *Daphnia magna* toxicity tests due to lowered pH from snow melt). We must focus on problems that really matter. To do otherwise is foolish, and for those of us who have children, criminally so. If, as I have previously recommended (Chapman, 1989b), laboratory acute (lethal, sublethal) and chronic (full-life cycle or the most sensitive life-cycle stages) tests together with ambient effects testing show that there is no problem, believe these data. This is just common sense. It is impossible to prove that an effect of one sort or another might not occur under just the right (sometimes improbable) combination of circumstances. But, to quote Aristotle: "It is the mark of an instructed mind to rest satisfied with the degree of precision which the nature of the subject permits and not seek an exactness where only an approximation of the truth is possible."

Opportunity/Pitfall #6

Opportunity: Prioritize actions and responses

Toxicity testing should be conducted on an appropriate, limited battery of species, end-points and exposure routes. If this is done and the resulting data are interpreted holistically, we have the clear opportunity to prioritize areas, industries, inputs, and human activities of most concern. Then, we can determine which problems merit remedial and/or preventive measures, the relative level of effort we should expend, and justify such to managers, politicians and the public. This and previous opportunities can provide the evidence by which human beings, with their homocentric view of the world, will demand and get just as clean an environment as if we all had a biocentric view of the world (assuming, of course, that we did not want to live in caves, but rather to enjoy the benefits of industrialised civilization).

Pitfall: Don't compartmentalize the environment

We cannot prioritize actions or even problems if we do not think and act holistically. Most human environmental endeavours are divided along unnatural lines; for instance, water, land and air are generally treated separately by regulators. The NIMBY (Not In My Back Yard) syndrome is alive and well not only in neighborhoods but also in bureaucracies, legal arenas (e.g., regulations), and scientific organizations. For instance, administrators objecting to in-water disposal of dredged material often recommend land-based disposal. And they generally do so without regard to the potential problems this would entail, and also often without consulting their counterparts responsible for the very lands which would receive this material. I would be surprised if anyone disagreed with the motherhood statement that multidisciplinary research is needed, but research funding agencies such as the National Sciences and Engineering Research Council (NSERC) generally do not fund such research.

Opportunity/Pitfall #7

Opportunity: Obtain complex answers, where necessary

The environment which comprises our world is a complex system. It is unlikely that simple "yes/no" answer will apply to such complexity. I was once asked by group of miners whether copper is toxic. They could not understand how I could not simply say "yes" or "no" until I explained that a copper penny will kill fish in soft water but will have no effect in salt water. Then they finally understood that copper is not always toxic and the only possible answer is "maybe". Information on the conditions resulting in toxicity and biological effects is generated by toxicity tests, and is essential for both generic and site-specific environmental regulation.

Pitfall: Don't necessarily agree to simple "yes/no" answers

Copper is generally considered a toxicant (a "yes", not a "maybe") and is included in many lists of chemicals of concern. This type of unnecessary oversimplification of complex issues is not helpful. There are many examples of such regulatory oversimplifications which lead to wastage of precious resources and talent addressing non-problems. Even worse, such oversimplifications, when they are inevitably revealed, confuse the public and lessen their trust in professionals. Regulatory requirements, based on adversarial legal approaches, encourage and, in many cases, can only deal with simple "yes/no" answers. This is not science, nor is it useful. Although toxicity tests can give simplistic answers (e.g., fish die or they do not), as is obvious with the example of copper, they can also give us complex but realistic answers. We forget the 16th century wisdom of Paracelsus at our peril: "All substances are poisons, there is none which is not a poison. The right dose differentiates a poison from a remedy."

Opportunity/Pitfall #8

Opportunity: Good, trustworthy data through QA/QC

Quality Assurance (QA) refers to externally imposed requirements; Quality Control (QC) refers to internally imposed requirements. QA/QC requirements for toxicity tests include the use of controls (positive [=known toxicants to ensure a response occurs when it should] and negative [non-toxic exposure conditions to ensure a response does not occur when it should not]), healthy test organisms, maintenance of proper testing conditions and records, blind testing (where possible), reference samples (where appropriate, e.g., reference sediments in sediment toxicity tests to ensure that responses are not affected by sediment characteristics such as grain-size). Both QA and QC assure reliable and trustworthy data from toxicity tests, and help explain any anomalies unrelated to the substance being tested.

Pitfall: The rules are not the end in themselves

I remember a talk given by Dr. Don Mount of the U.S. EPA at the 1989 Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in Toronto, Ontario. Dr. Mount described how QA/QC rules, if enforced blindly, would require failure of a pass/fail fish bioassay (consisting of a test container with 100% effluent and a control container with clean water), in which the control fish died but the 100% effluent-exposed fish all lived. Since the objective of this test is to determine whether or not effluent kills fish, not whether control water kills fish, any test in which the fish live in the effluent can only lead to the conclusion that the effluent is not acutely lethal to these test fish. Clearly, although QA/QC are necessary, so too is common sense.

Pitfall: Toxicity tests include uncertainty

A common regulatory failing is to consider the results of toxicity tests as being absolute. They are not. Toxicity tests do not give a single definitive number, but rather a range of values (Chapman, 1989b, 1991). Recognition and acceptance of this uncertainty are necessary for effective regulation which avoids either false positives (determining there is an effect where none exists) or false negatives (determining there is no effect when one exists).

Opportunity/Pitfall #9

Opportunity: Use statistics to determine differences

Statistical analyses serve to determine differences between control and/or reference results and test results. Statistical differences provide the starting point for determinations of environmental significance (or lack thereof) by providing a non-subjective basis for determining differences.

Pitfall: Statistics do not indicate biological significance

Statistics, used with laboratory toxicity tests, serve to determine differences between numbers generated outside the natural environment. By themselves they do not indicate biological significance. As Barnthouse et al. (1989) have noted: "...given extremely precise data, very small and biologically inconsequential changes might be found statistically significant." However, many regulators and regulations wrongly equate statistical and biological significance.

Opportunity/Pitfall #10

Opportunity: Ability to make a clear decision

In many cases, "worst case" toxicity testing should lead to clear decisions. Admittedly, such decisions may be complex rather than simply "yes/no". But, in many cases, toxicity test results will, at least, very clearly focus further work to determine whether (or not) there is any biological significance in the real environment. Good environmental science involves making decisions based on scientific facts and best professional judgment; such decisions allow society to make the best measured, rational choices.

Pitfall: Refusal to make a decision

The CYA (Cover Your Ass) syndrome is endemic in bureaucracies; regulatory bodies are no exception. I have coined a phrase to describe this situation, taken from personal development instruction, where one is urged to make clear decisions based on whether one would rather be right (e.g., win an argument) or be happy (e.g., maintain a personal relationship). Basically, what the following phrase means is that we need to be clear in our ultimate goal of a clean environment:

> It is easy to "be right"; it is much harder to "do it right".

I was recently in a situation where a particular individual, rather than making a decision on the data, elected to conduct more and more esoteric studies. When I protested, this individual loftily told me that they were working to protect the environment, and left the clear impression they felt I was only in it for money. The reality was and is that such individuals are not protecting the environment as they may think they are; they are responsible for a loss of focus on real problems. Their actions, resulting in inappropriate diffusion of effort, are among those that ultimately injure the environment.

Pitfall: Misusing facts

There is a second and shameful major pitfall to this last opportunity. Most scientists are aware that many environmental groups prefer what they call "good lies" to what they consider "bad truths" (Pearce, 1991). Reprehensible as such actions are by lobby groups, it is much worse when scientists in positions of influence misrepresent facts. This can result in both unwarranted focus on "problems" that may not merit such focus and a general loss of trust in scientists. Strauss (1991) documents an example, when "politically compromised scientists who work for the International Joint Commission" caused an unnecessary near panic among breast feeding mothers over PCBs because (Dr. Ross Hall, co-chairman of the IJC Health Committee): "IJC is in some ways an advocacy group". Frankly, I find such distortion of the truth repugnant. Don't you? Let's not be like Chicken Little and tell people that "The sky is falling!" unless it really is. Remember, the boy who "cried Wolf" unnecessarily was finally eaten by the wolf.

RECOMMENDATIONS

The following recommendations follow from the above opportunities and pitfalls:

Recommendation 1: Test "worst case"

If we always test "worst case" (test material that is most likely to be contaminated and toxic, tests and species that are most sensitive and responsive) then we will be, at the very least, able to use toxicological data to screen non-problems from real problems. Granted, most of the environment will fall into an intermediate "gray zone" (cf. Figure 1), but focused studies based on initial toxicity data will help clarify such intermediate situations. As part of such tests, we also need to clearly differentiate chemical from non-chemical (e.g., physical) toxicity (cf. Chapman et al., 1987) for purposes of regulatory control and remediation.

Recommendation 2: Prioritize concerns

Death is worse than life. If organisms are dying in great numbers, this is much worse than the possibility of "subtle [but unproven] long-term effects". Deal with the immediate, clear problems first. When you are in a burning house your immediate worries are burning alive and suffocating from the smoke, not heat prostration. And, most importantly, educate the public so they are "bought into" any prioritization. Perception in terms of societal needs and wants must be balanced by the reality of scientific facts and knowledge.

Recommendation 3: Think and act holistically

Remember that we are trying to protect ecosystems. Do not compartmentalize. Reductionism alone is not the answer. Testing at lower organizational levels provides a greater opportunity to be proactive but also provides a greater chance of false positives (with consequent wastage of effort, talent and resources on non-problems). Testing at higher levels tends to be reactive (generally after the fact) but realistic. Clearly, we need both holistic (top down) and reductionist (bottom up) approaches. Ideally, integrative assessments (Chapman et al., 1992) provide the best information for decision-making and resource allocation.

Recommendation 4: Clearly define the ultimate goal

For example, if protection of a fishery is the ultimate goal, why are we protecting it? For human consumption? If so, why is it permissible to fish a lake to death but not to add chemical contaminants that cause, in some cases, negligible effects? This conundrum is one I recently encountered and which no regulators seem ready to deal with.

Recommendation 5: Perform constant reality checks

Reality checks can be simple questions: "Why am I doing this?" "Is this really useful?" The simplest and yet most incisive question of all, which we need to ask both of toxicity tests and of ourselves is: "So What?". Basically, we need to remember the words of the comic strip character Pogo: "We have met the enemy and they are us".

Recommendation 6: Cooperate, question and explain

All too often those working for government environmental agencies are seen as "white hats" (i.e., heroic) while those working for industry are seen as "black hats" (i.e., villainous). I have had the personal experience, as a consultant, of being regarded very differently by the same individuals depending on whom I was working for. The reality is that there are only "us", and we do best to talk to each other openly and cooperatively. Burden of evidence and best professional judgement benefit from as much professional input as possible, no matter the colour of our "hat". Only in this way can we hope to address the major problems, dismiss the nonproblems, and prioritize intermediate problems.

Recommendation 7: Perform focused interdisciplinary research

Interdisciplinary toxicological research is badly needed on: key functional processes; key taxa; and, response, adaptation and recovery. I use the term "key taxa" rather than "key species" because many of the recognized species are the result of taxonomic publications, and may not reflect reality (e.g., see Chapman and Brinkhurst, 1987). We need to both educate (real problems, what needs protection and why) and be educated by the public (what do human beings in general want to protect?). Only then can we really focus such research to meet human needs rather than wants. This homocentric approach is, I believe, the likeliest to obtain support from developing as well as developed countries.

Recommendation 8: Use a "burden of evidence" approach

A burden of evidence approach is necessary because adequate knowledge of cause/effect is presently lacking. Such an approach requires best professional judgement such that:

- conclusions from individual components are considered relative to each other (e.g., the significance of laboratory toxicity tests must be considered relative to the real environment)
- different viewpoints assist in determining possible mechanisms.

Toxicological data are only part of the answer to the bigger question of environmental significance (or lack thereof). The U.S. EPA (1991) is clear on this point: "To help restore and maintain the biological integrity of the Nation's waters, it is the policy of the Environmental Protection Agency (EPA) that biological surveys shall be fully integrated with toxicity and chemical-specific assessment methods in state water quality programs..."

FINAL WORDS

From a homocentric viewpoint (Chapman, 1991): "...excessive over-protection is as undesirable as is under-protection, if human needs are to be met." Further, compared to the prospect of global climate change, for which scientific evidence is rapidly becoming compelling (e.g., Cook et al., 1991), or even habitat loss (Chapman, in press), many of the toxicological "problems" we deal with (e.g., contaminated sediments) are inconsequential. My greatest professional and personal concern (=fear) is global climate change which, like many of our concerns, is only a symptom of the two biggest problems facing humanity at present: overpopulation (in developing countries) and energy consumption (in developed countries). I am not advocating ignoring real problems of a toxicological nature. Rather, I am advocating putting our combined efforts, as responsible scientists, into the most compelling problems in our discipline. I am also advocating a cessation to the furthering of individual and collective purposes which may be cloaked in the banner of environmental protection but which are often only selfish personal goals.

The "best" (=most appropriate, most environmentally protective) decisions are based on best professional judgement, which takes into account: facts, intuition, background knowledge, the actual site, and experience. Such an approach is necessary if we are to avoid either over- or under-protection (cf. Figure 1). The ultimate bottom line to using toxicological data in a regulatory context, is to use common sense. One thing I know about common sense - it isn't so common. As a result, our regulatory arena is becoming increasingly rigid and proscriptive. This results in much lessened flexibility, which does not help us achieve our ultimate goal of environmental protection. We do need enforcement in regulatory agencies. But, much more, we need more good science and good scientists who are willing to loudly and clearly tell the truth, the whole truth and nothing but the truth!

ACKNOWLEDGMENTS

This paper was written at the request of Richard Scroggins of Environment Canada, Ottawa, who provided the title of this Plenary presentation and funds for my attendance at the Aquatic Toxicity Workshop. Beth Power of E.V.S. Consultants provided useful review comments. However, neither is in any way responsible for the contents of this paper, which are solely my responsibility.

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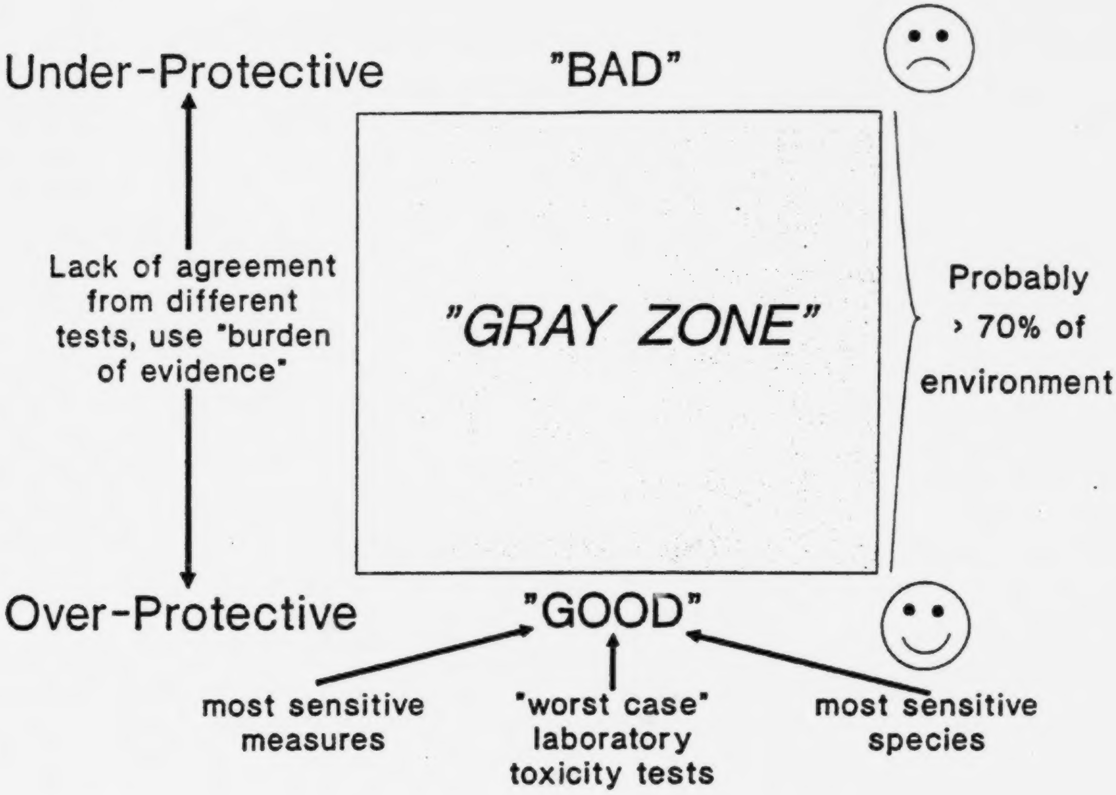
Under-Protective
"BAD"
Lack of agreement from different tests, use "burden of evidence"
"GRAY ZONE"
Probably › 70% of environment
Over-Protective
"GOOD"
most sensitive measures
"worst case" laboratory toxicity tests
most sensitive species

Figure 1. Distinguishing "good" from "bad" environmental conditions. Adapted from Chapman (1991).

APPLICATION OF AQUATIC TOXICOLOGY IN ENVIRONMENT CANDA'S REGULATION PROGRAM. G. Allard, Environment Canada, Technology Development Branch, Hull, Québec.

Acute fish toxicology bioassays have formed a part of Environment Canada's regulatory program since they were first incorporated into the Fisheries Act Pulp and Paper Liquid Effluent Regulations in 1971. Sine that time, many advances in aquatic toxicity have been made, but an equivalent use in their use as a regulatory control parameter has not occurred. This presentation provides a manager's perspective on the value of toxicity testing to Environment Canada's regulatory programs and offers suggestions on how to make better use of this valuable tool.

BIOAVAILABILITY OF METALS IN THE AQUATIC ENVIRONMENT: ANALYTICAL, GEOCHEMICAL AND BIOLOGICAL ASPECTS. P.G.C. Campbell, Université du Québec, INRS-Eau, Ste-Foy, Québec.

The bioavailability of metals introduced into the aquatic environment, and thus their impact upon aquatic life, will depend to a large extent on their speciation (i.e. their partitioning among various physical and chemical forms or "species"). The Free Ion Model (FIM) of metal toxicity, as developed from laboratory experiments over the last 10+ years (e.g. Morel 1983), predicts that the bioavailability of a given metal (as a micronutrient and/or as a stressor) will be proportional to the free ion activity of the metal in the exposure medium. The strengths and weakness of the FIM will be discussed in this presentation, with examples drawn from work in our laboratory and elsewhere. Particular emphasis will be accorded to the applicability of the FIM approach to the field.

Principles of Aquatic Chemistry, Wiley-Interscience, pp. 300-309.

SESSION 1A

ABIOTIC FACTORS WHICH INFLUENCE CONTAMINANT TRANSFER/FACTEURS ABIOTIQUE QUI INFLUENCENT LE TRANSFERT DES CONTAMINANTS

CHAIRPERSONS/PRÉSIDENTES
Chris Metcalfe and Gail Krantzberg

CHARACTERISTICS OF COHESIVE SEDIMENTS DURING SETTLING.

Y.L. Lau,
Environment Canada, National Water Research Institute, Burlington, Ontario.

INTRODUCTION

Erosion and deposition are two of the key physical processes governing the fate of sediment-bound contaminants in the aquatic environment. Because the degree of affinity of contaminants can vary with the size of the sediment, knowledge of the characteristics of the sediments during settling is very important. This is especially true in the case of cohesive sediments which can flocculate and settle at rates very much different from that of the primary particles.

Modelling the transfer of these contaminants through the aquatic environment requires knowledge of the sediment transport. This includes information on settling velocity, critical shear stress as well as their variation with different size fractions. In most models, an average settling velocity is used for all the suspended material. Some more sophisticated models consider the transport of different size fractions separately. For such models, assumptions have to be made as to how the settling velocity varies with the size fractions. For example, Mehta and Lott (1987) assumed that the settling velocity has the same distribution as the initial concentration. Some models assume the settling velocity to be proportional to the concentration, while in many cases, the settling velocity is considered constant throughout the settling process. Measurements of changes in concentration and size distribution during the settling of fine sediment suspensions in a circular flume are reported herein. These data provide some useful insight into the process of settling of cohesive sediments and show that some of the assumptions mentioned above are not very valid.

EQUIPMENT AND METHOD

The experiments were carried out in an annular flume which has an outside diameter of two metres and a channel 20 cm in width. A top ring which fitted inside the channel could be lowered so that it just touched the water surface. Flows were generated by rotating the ring at various speeds.

Two different sediments were tested. The first was a kaolinite clay with a mean diameter of about 6.8 microns. The second was a sediment obtained from the Nith river in Southern Ontario, containing a combination of kaolinite and mortmorillonite and having a mean diameter of about 12 microns. The kaolinite clay was tested using distilled water as well as a 2% salt solution as the fluid medium, while the river sediment was tested using water drawn from the river. Flow depths were kept relatively constant at about 8 cm..

Before beginning an experiment, the water-sediment mixture was thoroughly mixed first by a mechanical mixer and then by running the top ring at a high speed. The ring was then slowed to the desired speed to begin the experiment. Samples were withdrawn isokinetically from the flume through a 5 mm diameter sampling tube. Samples were taken at intervals until the concentration reached and remained at a constant equilibrium value. Following the completion of one experimental run, the sediments were completed resuspended and the experiment was repeated using another speed for the top ring, i.e., a different shear stress.

The concentration of each sample was determined by filtration, drying and weighing. The grain size distribution of the dispersed samples, i.e., the primary particle distribution, was determined using a Malvern Particle Size Analyzer (model 2600 c) which operates on the light diffraction principle (Fraunhoffer diffraction). Complete details of the instrument can be found in Weiner (1984). The output of the Malvern Particle Size Analyzer consists of percentage of particles by volume in different size fractions and a cumulative size distribution curve from which median diameter and other statistics of the size distribution were calculated.

The concentration of each particular size fraction in the suspension was calculated using the data on total concentration and size distribution. Thus the change in concentration of each size fraction with time was obtained. This data was then used to calculate the effective settling velocity of each size fraction.

RESULTS AND DISCUSSION

The pattern of the decrease in concentration during settling is similar to that found by previous investigators, e.g., Mehta and Partheniades (1975) and Lick (1982). As deposition takes place, the suspended sediment concentration first decreases and then becomes constant at some value which has been termed the equilibrium concentration. The ratio between the equilibrium concentration and the initial concentration depends on the bed shear stress generated by the flow. This ratio decreases when the bed shear stress is reduced as more sediment is able to deposit.

The data show that the median diameter, D_{50}, of the material left in suspension (in dispersed state) also behaves in the same manner as the concentration, i.e., it decreases with time before levelling off at a constant value. As shown in Fig. 1, the curve of D_{50} versus time is very similar to the concentration versus time curve. The median diameter becomes constant at about the same time the concentration becomes constant. This behaviour can be found for every test run. The data presented in these figures are from the kaolinite/distilled water tests. However, essentially the same results were found for the kaolinite/salt solution and Nith River sediment tests. Size distribution curves for a typical test run are shown in Fig. 2. From the above results it is clear that the material which has deposited does not have the same size distribution as the initially suspended material.

After reaching equilibrium, the suspension has a median diameter, D_{eq}, which is finer than the D_{50} of the original suspension. The values of D_{eq} for the various runs are plotted against the bed shear stress in Fig. 3. It can be seen that D_{eq} decreases steadily as the bed shear stress decreases. The fact that the size distribution changes as settling progresses, with D_{50} decreasing, implies that flocs are not composed of particles from all size classes in the same proportion as the original

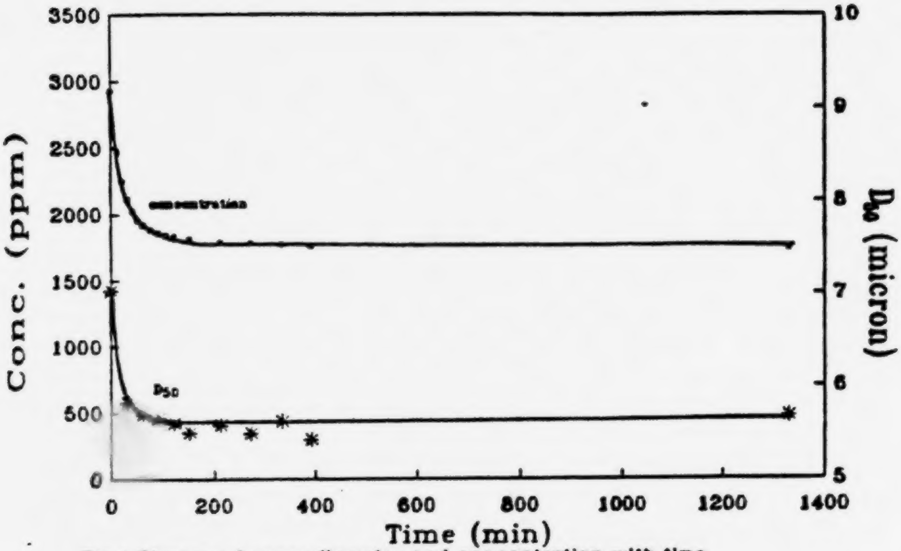

Fig.1 Change of mean diameter and concentration with time.
Ring speed = 8 rpm. Bed shear stress = 1.72 dyne/cm².

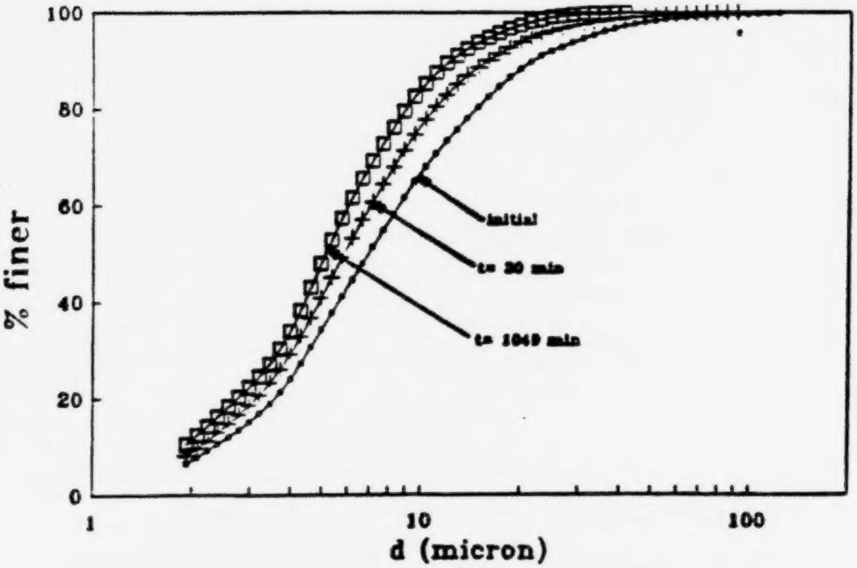

Fig. 2 Change in size distribution with time. Ring speed = 7 rpm.

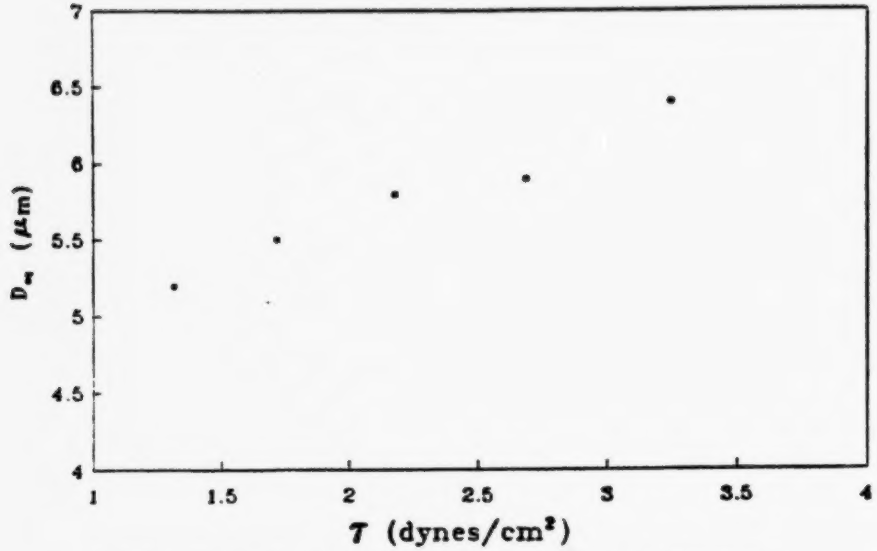

Fig. 3 Effect of bed shear on mean diameter at equilibrium.

suspension, as inferred by Kranck (1980) from experiments in settling columns. This result suggests that the coarser particles are associated with stronger flocs which can withstand the higher shear near the bed and are able to deposit, while the flocs containing more of the finer material get broken up near the bed and are resuspended. This is also consistent with the result that D_{eq} increases as the shear stress increases. When the shear stress is increased, some of the flocs which previously were strong enough to settle are now also broken up and resuspended. As these contain more larger particles, the distribution in the suspension becomes coarser. Therefore, contaminants which are associated with certain size fractions may be transported through a river system at certain times but may get deposited if the flow and turbulence conditions are altered.

Fig. 4 gives the size distribution histograms from one of the runs. Shown are the initial distribution, when the total concentration was 3000 ppm, and the distribution at t= 92 min. when the settling had reduced the total concentration to 1862 ppm. It can be seen that the concentration of every size fraction had decreased, indicating that material from every size range had deposited. Also shown in the figure is the distribution obtained from a numerical model assuming single grain settling, starting with the same initial size distribution and total concentration. The computed size distribution when the total concentration had dropped to 1862 ppm is shown as a comparison. In this case, there is practically no deposition of material smaller than 3 microns. The deposition of the finer fractions is also much smaller , while a much larger amount is deposited from the coarser fractions. Thus it is evident that the deposition of the finer fractions in the flume experiments was the result of their ability to aggregate into flocs. This illustrates the important role played by the flocculation process, whether it is caused by electrochemical forces or by biological factors. If there were no flocculation, the finest fractions would not have deposited at all.

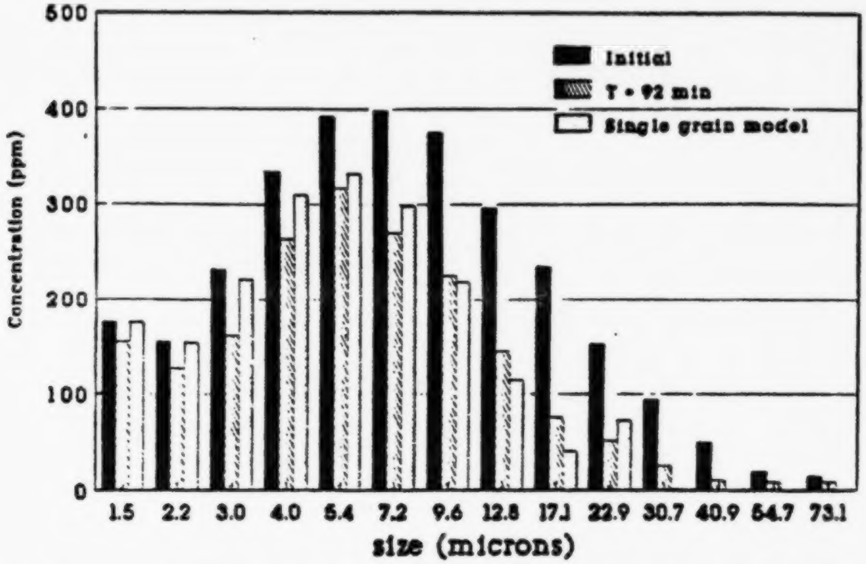

Fig. 4 Measured distributions and computed distribution for single grain settling.

Fig. 5 is a plot of the average settling velocity of some of the size classes against time for a run in which the shear stress was low enough for all the material to eventually deposit. It can be seen that there is a substantial increase of settling velocity with size in the early stages. However, the decrease is much more rapid for the larger sizes with the result that the settling velocity becomes practically the same for all sizes as time goes on. The same trend is found for all the other runs. Therefore, the assumption of constant settling velocities can lead to large errors.

It is fairly well known that the overall settling rate of a sediment suspension is an increasing function of the total concentration, provided that the concentration is not so high that hindered settling takes place. In their model for cohesive sediment settling, Mehta and Lott (1987) extended this idea to the distribution of settling velocity for the various size classes. Their assumption is that the distribution of the settling velocity, $\phi(W_i)$, is the same as the distribution of initial concentration, $\phi(C_{oi})$. The critical shear stress for deposition for each size is also related to its settling velocity and hence to the distribution of initial concentration. Their model also assumes that the sediment comprising those classes for which the critical shear stress for deposition is larger than the actual bed shear stress will all deposit while those classes with critical shear stress less than the bed shear will not deposit at all. However, present results show that deposition is evident for all size classes.

Fig. 6a shows the concentration histograms at the beginning and partway through one of the tests. Figure 6b shows the corresponding settling velocity histograms. There is little resemblance

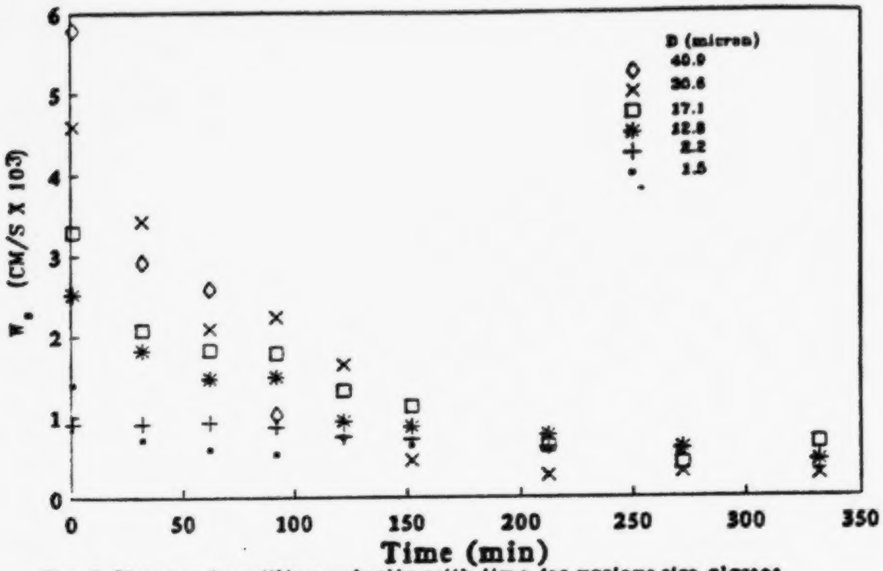

Fig. 5 Changes in settling velocity with time for various size classes. Ring speed = 5.8 rpm.

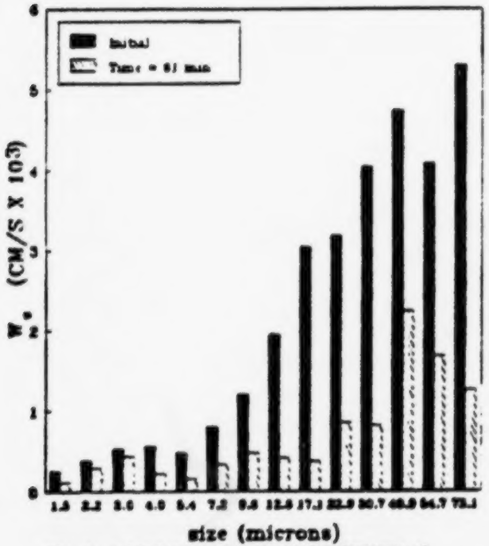

Fig. 6a Initial and subsequent distributions of settling velocity. Ring speed = 7 rpm.

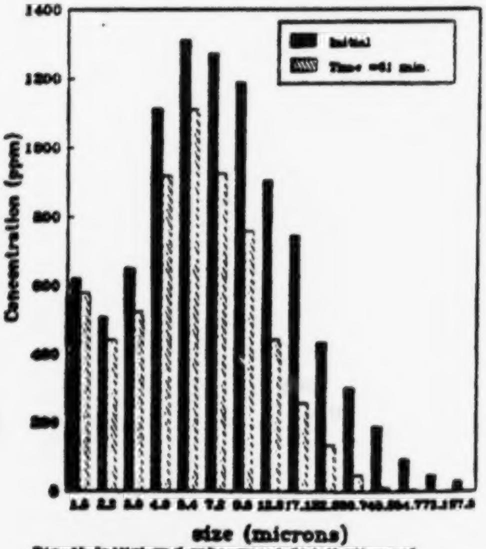

Fig. 6b Initial and subsequent distributions of concentration. Ring speed = 7 rpm.

between the concentration and settling velocity distributions at either times. This is found to be true for all the test runs. These results, plus the finding that deposition of all sizes occur during settling, certainly cast doubt on the assumptions used by Mehta and Lott.

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TRANSPORT OF PCBs IN THE OTONABEE RIVER-RICE LAKE SYSTEM.

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EXTENDED ABSTRACT

The section of the Otonabee River from Little Lake in the City of Peterborough, Ontario, downstream to Rice Lake has been contaminated with PCBs as a result of a history of accidental discharges into Little Lake from various industries in Peterborough. Studies at Trent University over the past few years have been directed at determining the extent of PCB contamination in this system, as well as determining the mechanisms by which PCBs are transported downstream.

Research on the fate of PCBs in aquatic systems has shown that these hydrophobic organic contaminants are rapidly adsorbed onto suspended particulates and deposited in bottom sediments when introduced into aquatic systems (1). Both physical and biological disturbances increase the partitioning of organic contaminants back into the water column (2). Desorption from contaminated sediments has been identified as a major source of PCBs in a riverine system (3). However, other studies have shown that transport in association with suspended sediments is a major vector for downstream movement of hydrophobic chemicals in rivers (4,5). Studies were conducted between 1987 and 1991 to determine the quantities and mechanisms of PCB transport from Little Lake down the Otonabee River to Rice Lake.

Concentrations of total PCBs (total of 19 congeners analyzed) in surface sediments within the Otonabee River-Rice Lake system varied from a high of 5.9 µg/g (dry wt.) in Little Lake (Station 2), to 0.4 µg/g in central Rice Lake (Station 7). The congener patterns in the sediments are remarkably consistent at all stations within the system, with the dominant congeners being the trichlorobiphenyls 31(28), the tetrachlorobiphenyls 52 and 49, and the pentachlorobiphenyls 101, 110 and 118. These congeners reflect historical contamination of the system with a mixture of commercial Aroclors, including Aroclor 1242, 1248, 1254 and 1260.

The total burden of PCBs in sediments within the 13.7 ha depositional zone of Little Lake was calculated as 37 kg for the top 6 cm ("active layer") of contaminated sediment. Bottom sediments in Little Lake are presumeably the major source of PCB contamination in the system at present, since discharges of PCBs from industry into Little Lake have not been detected since 1985, and there are no known sources of PCBs downstream. However, there is some evidence that small amounts of PCBs are still released from stormwater outlets discharging into Little Lake (6).

In a laboratory study to measure the "release" of PCBs from contaminated Little Lake sediments, the mean flux of PCBs (total of 19 congeners analyzed) was 0.9 $\mu g/m^2/d$ from a 6 cm layer of sediment with an average PCB concentration of 1 µg/g. PCBs dissolved in the water comprised the majority (65%) of the flux from sediment. By extrapolating these PCB release data to Little Lake, we would expect 12.3 mg of PCBs per day (total of 19 congeners) to be released from the active layer of the depositional zone, and we would expect that desorption into the dissolved phase would be the primary mechanism for PCB release.

The concentration of PCBs (total of 19 congeners) measured in the water column below Little Lake (Station 2) was 3.9 ng/L dissolved in water and 8.0 ng/L adsorbed onto suspended particulates. In contrast, concentrations upstream of Little Lake (Station 1) were 0.7 ng/L dissolved in water and 0.4 ng/L on suspended particles, respectively. Assuming a mean discharge rate for the Otonabee River at Little Lake of 85 m^3/s, this corresponds to a total outflow from Little Lake of approximately 8.8 g of PCBs per day and a total inflow into Little Lake of approximately 0.8 g of PCBs per day. To provide the observed increase in outflowing PCBs (8.8 - 0.8 = 8 g/day), the depositional zone of Little Lake would have to release 592 $\mu g/m^2/d$ of PCBs; a value far greater than the laboratory estimates of PCB release rates (0.9 $\mu g/m^2/d$), and greater than release rates determined in other laboratory or mesocosm studies (3,7,8,9).

The disparity between PCB release data estimated by mass balance and laboratory models for Little Lake can only be explained by: i) Higher than predicted desorption of PCBs from sediments into the dissolved phase, ii) Release of PCBs by physical resuspension of sediments, iii) Continued direct discharges of PCBs into Little Lake. Since the latter alternative seems unlikely, we investigated the possibility that large amounts of PCBs are transported downstream in the dissolved phase or in association with suspended sediments. As an initial
approach to this problem, we used freshwater bivalves, Elliptio complanata as a biomonitoring organism to investigate the dynamics of PCBs.

It is expected that highly chlorinated PCB congeners (hydrophobic) would remain adsorbed to suspended sediments, and less chlorinated congeners (hydrophilic) would tend to desorb into the aqueous phase. PCBs partition into bivalves through direct uptake from water or through ingestion of contaminated particles (10, 11). Bivalves were suspended in cages within the water column of the river system, where they were in a position to take up PCBs dissolved in water or adsorbed onto suspended particulates. It was hypothesized that congener patterns in the clams would give an indication of the major vector for PCB transport in the river system.

Introduced bivalves at Staions 1 through 6 showed a pattern of increasing total PCB concentration (total of 19 congeners) with distance downstream. This was unexpected, since concentrations in sediments decrease with distance down the river system. Congener specific data indicated that the increase in PCBs with distance downstream could be mainly attributed to higher concentrations of lower chlorinated congeners in the downstream bivalves. These data indicate that concentrations of PCBs dissolved in water may increase with distance downstream; primarily through the release of hydrophilic, less chlorinated compounds from sediments and/or suspended sediments.

In summary, the fact that PCB patterns and concentrations in introduced bivalves change with location in the river suggests that this process is governed by complex interactions in the partitioning of PCBs between particulate and aqueous phases. PCBs initially adsorbed onto particulates in the upper reaches of the system may desorb into the aqueous phase at downstream locations. The high rates of PCB release from sediments determined by mass balance calculations suggest that, at least in river systems, these processes may be underestimated by laboratory experiments. Future work in this system will focus on the direct measurement of the sediment-associated transport of PCBs.

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BIOAVAILABILITY OF TRACE ELEMENTS ASSOCIATED WITH PARTICULATES.

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Aquatic animals are exposed to trace elements through both solution and food. Most trace element criteria are based upon solute exposures; and many more studies have considered uptake from solution than have considered uptake from food. This approach has been justified by assumptions that: 1) Trace elements are only biologically available when they are in solution; and 2) All other exposure pathways are in equilibrium with and predictable from concentrations of the bioactive form of the trace element. The assumption of thermodynamic equilibrium does not apply to many elements; nor does the assumption that elements are only available from solution.

Recent studies have shown that most proposed Se criteria will substantially underprotect estuarine communities because they are based solely upon solute Se availability (toxicity) to macrofauna. Virtually all Se transfer to the estuarine bivalve Macoma balthica occurs via ingestion of food. Diatoms accumulate substantial body burdens of Se when exposed to low concentrations (nM) of selenite. When M. balthica were fed diatoms that had been exposed to selenite, 86% of the ingested element was assimilated. Using established ingestion rates for this species, a bioaccumulation model predicted steady state concentrations of Se in M. balthica similar to the concentrations observed in nature. Uptake rates of selenite from solution, on the other hand, could not explain the level of Se observed in the tissues of animals from nature. Substantial contamination of the food web of Suisun Bay, California, occurs at nM concentrations of Se in water. Model results suggest this level of contamination can be explained by food web transfer, but not by the exposures from solution that occur in that system.

Pulse-chase investigations of element assimilation from food are one key to providing information critical to understanding trace element bioavailability. Recently such studies have shown that assimilation of Cr differed among two bivalve species because of the proportion of food each partitioned to its digestive gland during digestion. Uptake of Cr from bacteria was efficient (60%) in Potamocorbula amurensis, but neither P. amurensis nor M. balthica assimilated appreciable quantities of Cr when the element was bound to iron oxides. Thus if Cr is bound principally to iron oxides in nature, solute exposures are the most dangerous to these benthos. If bacteria are a source of Cr, uptake from food must be considered. Element assimilation in copepods appears to be simpler to predict than in molluscs, presumably because crustacean digestive processes are principally extracellular. The bioavailability of different elements to copepods ingesting diatoms is determined by the quantity of element that occurs in the cytosol of the diatom.

Guidelines to protect aquatic environments from the toxic effects of trace elements should be based upon understanding of both water exposures and particulate exposures. For some trace elements, solute exposures may be important. For others they are not. The relative importance these pathways must be determined for each element, especially those that we suspect are not at equilibrium in nature. Development of combined solute/particulate guidelines could be based upon the empirical results of bioaccumulation studies. For any given test species those studies would need to determine: assimilation efficiencies from predominant food types, feeding rate, uptake rates from solution, and efflux rates. Bioaccumulation models can then be employed to estimate concentrations in food or water that would result in tissue enrichment. Critical levels of tissue enrichment are either those dangerous to the organism itself, or those dangerous to organisms that feed upon the species of interest. Guidelines that consider food web transfer of contaminants, and that are verified by data from nature, might be simpler to implement and more realistic in their protection of nature than those proposed to date.

ROLE DE LA CHIMIE DU SOUFRE SUR LA REMOBILISATION DU MERCURE DANS LES SÉDIMENTS DU FJORD DU SANUENAY.

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RESUME

Durant plusieurs années, le fjord du Saguenay (Québec) a reçu de nombreux rejets anthropiques de polluants tels que métaux traces, organochlorés, HAP, etc. Les datations avec le ^{210}Pb nous ont permis de reconstituer la chronologie de l'industrialisation de la région. On retrouve des concentrations de Hg supérieures à 3 μg/g correspondant aux années 1965-70. La majorité de ces contaminants demeurent stationnaires dans le sédiment. Par contre, le mercure semble connaître une certaine remobilisation diagénétique. Ainsi, malgré des taux de sédimentation élevés et un arrêt des rejets de la principale source depuis près de 15 ans, les sédiments de surface de ce système demeurent encore contaminés (0,6 ppm Hg). L'étude de la chimie du soufre permet en partie d'évaluer le rôle des sédiments anoxiques sur la remobilisation possible du mercure. Les résultats préliminaires de cette partie dont des profils de soufre réduit dissous (H_2S, S_n^{2-}, RSH...) ainsi que d'espèces solides (FeS, FeS_2 et S^0) sont présentés. Les sédiments du Saguenay contiennent des concentrations anormalement élevées en monosulfures et particulièrement faibles en pyrite. La présence de processus réactionnels avec les espèces soufrées sera entre autres vérifiée.

INTRODUCTION

Le Fjord du Saguenay occupe une vallée glaciale à environ 160 km au nord-est de Québec (fig.1). La matière organique apportée par sa rivière est abondante (plus de 10% de la matière particulaire en suspension) et principalement d'origine terrestre (Pocklington et Leonard, 1979). Les taux de sédimentation sont rapides, soit près de 7 cm/an à la tête du fjord (Smith et Walton, 1980) et d'environ 0,4 cm/an dans le bassin profond (Gagnon et *al.*, sousmis). La présence importante d'industries riveraines, particulièrement une usine de chlore-alcali, a contribuée à un apport considérable de contaminants anthropiques organiques et inorganiques depuis près de trois décades (Loring et *al.*, 1983). Ces hauts taux de sédimentation en font un lieu ayant de bons enregistrements historiques des apports industriels depuis 1947, début de l'industrialisation.

Le devenir des métaux lourds est en partie contrôlé par la diagénèse de la matière organique durant l'enfouissement et leur distribution peut être modifiée par des processus de remobilisation dans les couches sub-oxiques et de réduction des sulfates. L'étude de la chimie du soufre peut permettre en partie d'évaluer le rôle des sédiments anoxiques sur la remobilisation possible du mercure. Ainsi la seconde partie de cette étude s'intéresse à la spéciation du soufre dissous et solide dans les sédiments anoxiques du Fjord du Saguenay. Des résultats préliminaires de distribution du Hg ainsi que d'espèces dissoutes et solides du soufre réduit seront présentés afin de visualiser certaines similitures possibles entre ces espèces concernées.

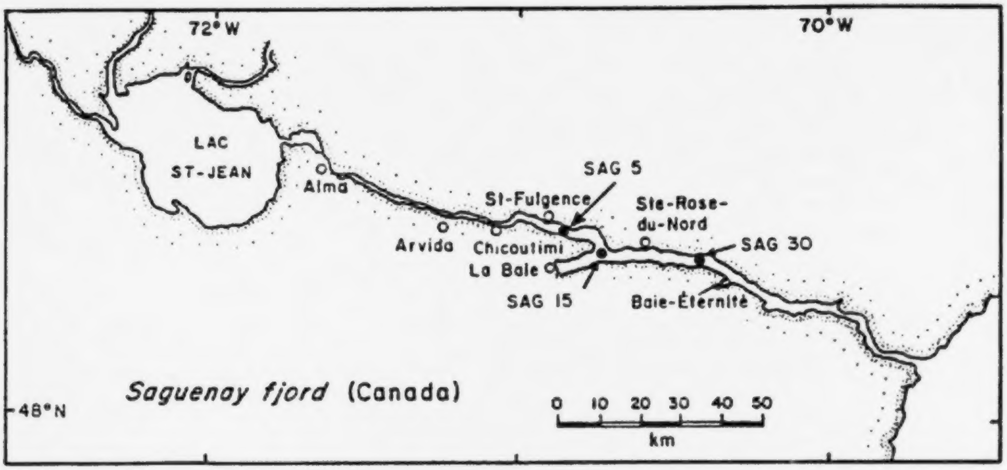

Figure 1. Emplacement du Fjord du Saguenay montrant les sites de carottage

METHODES

Les carottes de sédiment furent collectées à l'aide d'un carottier à boîte (0,12 m^2) et immédiatement sous-échantillonnées à l'aide d'une table à découper sous atmosphère d'azote puis congelées à -20 °C. Les eaux interstitielles furent obtenues à l'aide d'un pressoir à sédiment puis congelées à -20 °C. Trois stations ont été échantillonnées dont l'une à la tête du fjord (SAG-5; 90 m de profondeur), l'une à la jonction du fjord avec la baie des Ha!Ha! (SAG-15; 180m) et une autre dans le centre du bassin profond (SAG-30; 265m) (fig.1).

Les analyses du mercure total furent effectuées par spectroscopie d'absorption atomique avec réduction des hydrures suite à une digestion acide (Gobeil et Cossa, 1984). Les autres métaux furent analysés par spectroscopie d'absorption avec flamme (Al et Zn) et par spectroscopie atomique ICP avec plasma d'argon (Cr, Cu et Ni).

Les espèces dissoutes du soufre réduit telles $\sum H_2S$, $\sum S_n^{2-}$, $\sum RSH$, SO_3^{2-} et $S_2O_3^{2-}$ ont été dosées par titrage potentiométique sous atmosphère d'azote à l'aide d'une électrode sélective Ag-Ag_2S avec une solution de $HgCl_2$ aux pH 13 et 7 (fig.2). Cette méthode, adaptée de celle de Boulègue et Popoff (1979), permet des dosages avec de plus petites quantités d'échantillons de faibles teneurs en soufre. Le soufre élémentaire dissous, extrait avec le solvant $CHCl_3$, est dosé par spectroscopie UV à $\lambda=270$nm.

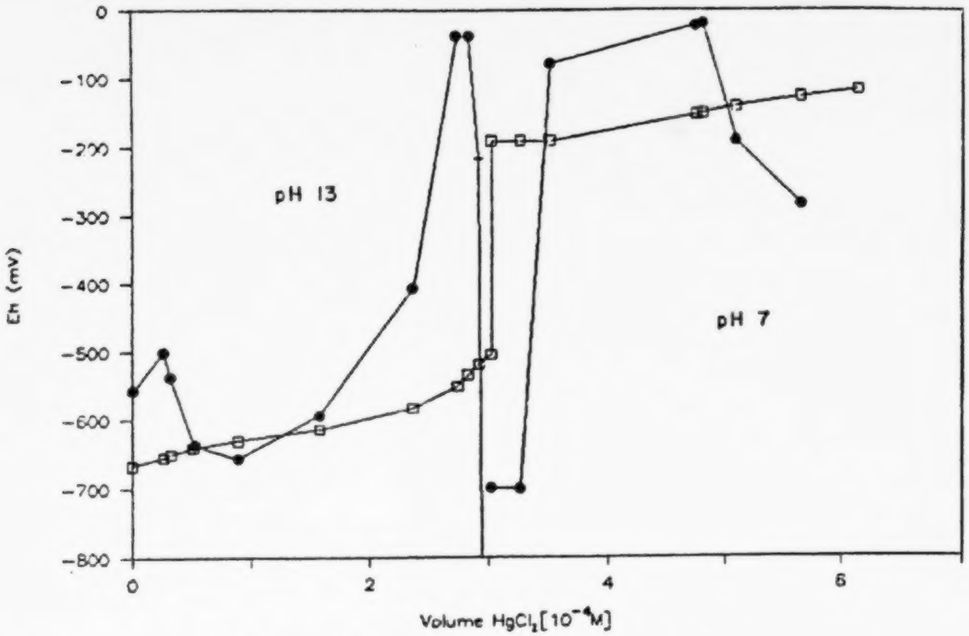

Figure 2. Courbes de dosage potentiométrique d'espèces dissoutes du soufre (□), (∂Eh/∂V:●)

Les espèces réduites du soufre de la phase solide du sédiment ont été analysés par diffusion de H_2S provenant des espèces ayant réagit de façon sélective. Le H_2S produit était capté dans des trappes d'acétate de zinc et mesuré par titrage iodométrique. (Hsieh et Yang, 1989). Une diffusion en milieu acide de 3 h était nécessaire pour le FeS. Une seconde diffusion de 48 h avec une solution acide de chrome réduit libérait les espèces FeS et FeS_2. A cette solution, un addition de N,N-diméthylformamide (DMF) et une distillation de 17 h libérait le soufre élémentaire.

Les carottes ont été datées par mesures de l'activité du ^{210}Pb déterminées à l'aide d'un compteur de particules α (Nittrouer et *al.*, 1979).

RESULTATS ET DISCUSSION

La figure 3 présente la distribution des rapports métal/Al pour le Cu, Cr, Ni et Zn. Les profils des métaux Cu, Cr et Ni ne présentent pas d'enrichissement et pourraient confirmer que les apports des 30 dernières années auraient été constants. Ces résultats sont en accord avec ceux publiés par Loring et *al.*(1983) et Barbeau et *al.* (1981) pour des échantillons prélevés à l'aide d'un carottier à gravité en 1976 et 1978 respectivement. Par contre les profils des rapports zinc/Al présentent certains enrichissements historiques, particulièrement dans la carotte SAG-15 où il y a une augmentation régulière de 1946 à 1976. On retrouve des concentrations allant de 88 à 122 μg/g et 105 à 133 μg/g dans les carottes SAG-15 et Sag-30. Le niveau pré-industriel moyen du Zn a été estimé à 85±20 μg/g (Barbeau et *al.*, 1981).

Tout comme le Zn, le mercure semble connaître le même profil de contamination. La figure 4 présente un net enrichissement en Hg avec des concentrations maximales ayant un ordre de grandeur plus élevé que celles de niveau pré-industriel. En dépit de la fermeture en 1976 de l'usine de chlore-alcali responsable des apports en Hg, les concentrations dans les sédiments de surface demeurent encore élevées.

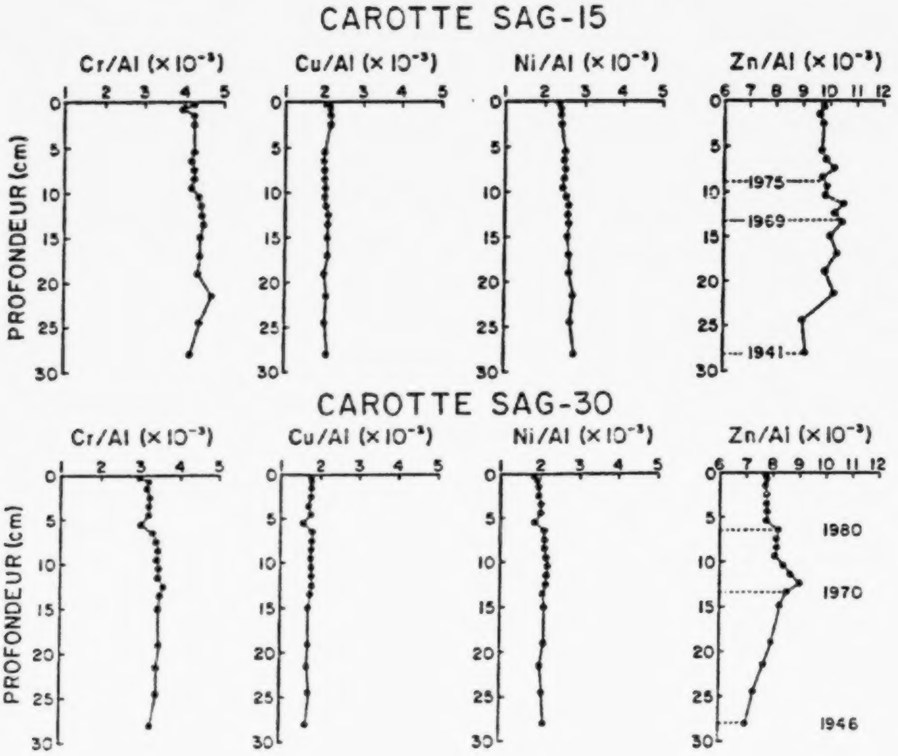

Figure 3. Rapports métal/Al du Cu, Cr, Ni et Zn dans les carottes SAG-15 et SAG-30

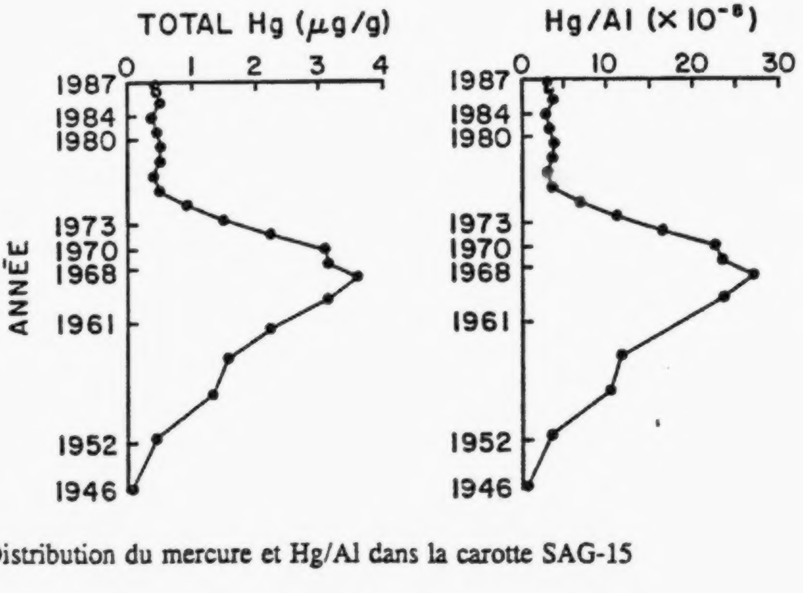

Figure 4. Distribution du mercure et Hg/Al dans la carotte SAG-15

Une des hypothèses intéressantes retenues afin d'expliquer la contamination des sédiments récemment déposés serait la remobilisation chimique possible du mercure dans les couches sub-oxiques du sédiments. Il a été intéressant de superposer notre profil de distribution en mercure avec celui fait par Gobeil et Cossa (1984) à partir d'une carotte prélevée en 1983 au même endroit avec les mêmes méthodes d'échantillonage et de sous-échantillonage et une méthode analytique similaire (fig.5). De façon générale, la distribution de 1987 semble être conservée puisque semblable à celle de 1983 avec un décalage de 4 ans. Ainsi en assumant un taux de sédimentation constante durant cette période de 4 ans, ces résultats indiquent une certaine remobilisation chimique du mercure des couches profondes du sédiment vers la surface.

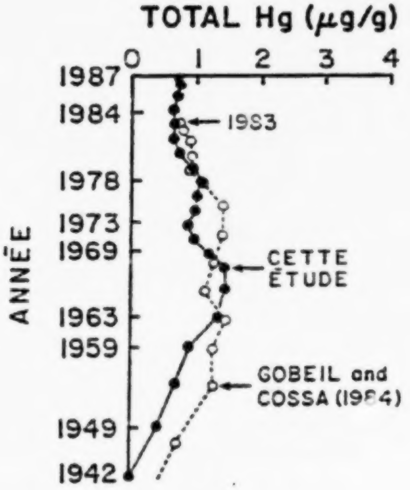

Figure 5. Distribution du mercure total et Hg/Al dans la carotte SAG-30 (Profil 1983 retracé de Gobeil et Cossa, 1984)

Afin de permettre d'évaluer cette possible remobilisation chimique du mercure dans les sédiments anoxiques du Fjord du Saguenay. Une étude plus approfondie à été entreprise sur la chimie du soufre réduit. La réduction des sulfates par les microorganismes est responsable de la présence d'espèces du soufre réduit dans les sédiemnts anoxiques. Toutefois, dans les 30 premiers cm des sédiments du bassin profond, les taux de réduction des sulfates avec des maxima de 1,5 nmol/cm^3/jour sont considérés comme faibles et seulement 3% de la matière organique serait dégradée via la réduction des sulfates (Edenborn et *al.*, 1987).

Pour la spéciation du soufre de phase solide, le monosulfure, la pyrite et le soufre élémentaire ont été dosés dans le sédiment. La figure 6 présente les profils de ces espèces à la station SAG-15. Les sédiments du Saguenay contiennent des concentrations anormalement élevéees en monosulfures de fer (FeS) et des concentrations particulièrement faible en pyrite (FeS_2).

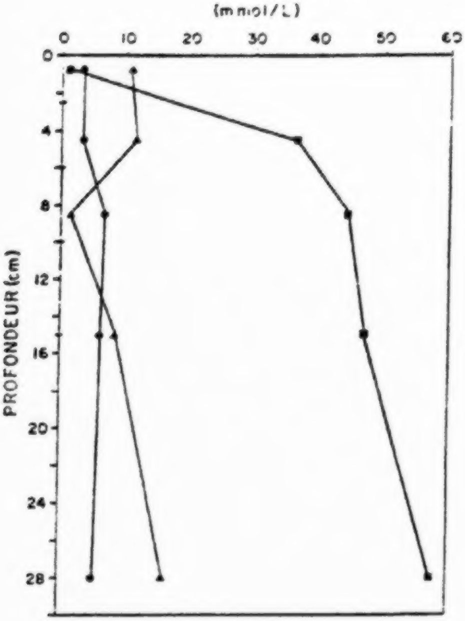

Figure 6. Distribution d'espèces du soufre de la phase solide à la station SAG-15. (FeS: ■ ; FeS_2: ▲ et S_8: ●)

Les espèces dissoutes du soufre réduit telles $\sum H_2S$, $\sum S_n^{2-}$, $\sum RSH$, SO_3^{2-}, $S_2O_3^{2-}$ et S_8^0 ont été dosées. La figure 7 présente une distribution assez dynamique de ces espèces dans la carotte SAG-5 située à la tête du fjord. Des concentrations maximales apparaissent au côté de la couche d'argile provenant de glissements de terrain. Ainsi des teneurs élevées en soufre organique réduit ($\sum RSH$) de près de 33 mmol/L sont obtenues. Dans cette zone, des concentrations maximales en soufre réduit tel $\sum H_2S + \sum S_n^{2-}$ (≈ 5 umol/L), de même qu'en soufre moins réduit tel S_8 (≈ 22 umol/L) et $SO_3^{2-} + S_2O_3^{2-}$ (≈ 15 umol/L) sont aussi rencontrées. La présence importante d'espèces intermédiaires du soufre réduit démontre une certaine dynamique dans le sédiment. La figure 8, présentant des profils d'espèces dissoutes de soufre réduit de la carotte SAG-30, démontre une similitude entre la distribution du mercure total dans le sédiment et la répartition des espèces dissoutes du soufre réduit.

CONCLUSION

Les apports anthropiques de Zn et Hg ont été confirmés par l'analyse de carottes et à l'aide d'une nouvelle datation au ^{210}Pb. Il a été démontré qu'il y a une possible remobilisation chimique du Hg dans les sédiments anoxiques et que le temps de réponse dans ces sédiments est très rapide.

La distribution d'espèces du soufre réduit contenues dans les sédiments sub-oxiques et anoxiques pourrait expliquer l'évolution temporelle des profils de Hg. La présence des processus avec le Hg de précipitation/adsorption sur le monosulfure de fer et la pyrite, de précipitation avec les sulfures d'hydrogène et d'augmentation de sa solubilité par complexation avec les polysulfures et les sulfures organiques disssous seront entre autres vérifiés dans la suite des travaux. Ainsi l'étude poursuivera dans cette approche afin de faire certains rapprochements entre le cycle du Hg et celui des espèces stables et métastables du soufre présentes dans les sédiments.

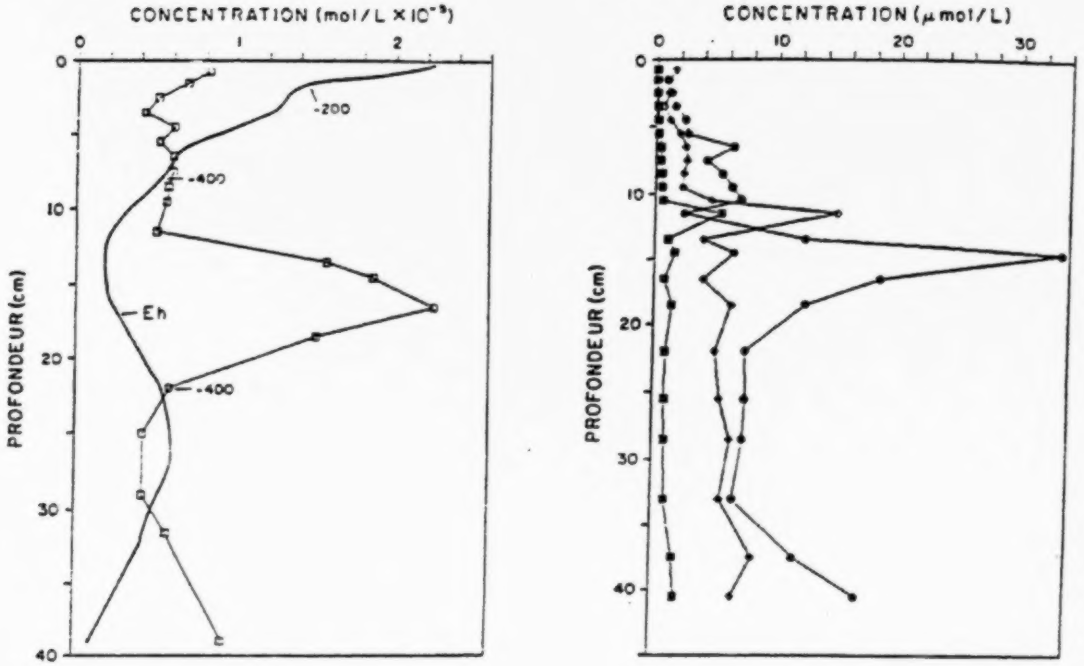

Figure 7. Potentiel rédox et profils de concentrations d'espèces dissoutes du soufre réduit dans la carotte SAG-5 ($\sum H_2S+\sum S_n^{2-}$:■; $\sum RSH$:●; $SO_3^{2-}+S_2O_3^{2-}$:◇ et S_8:□)

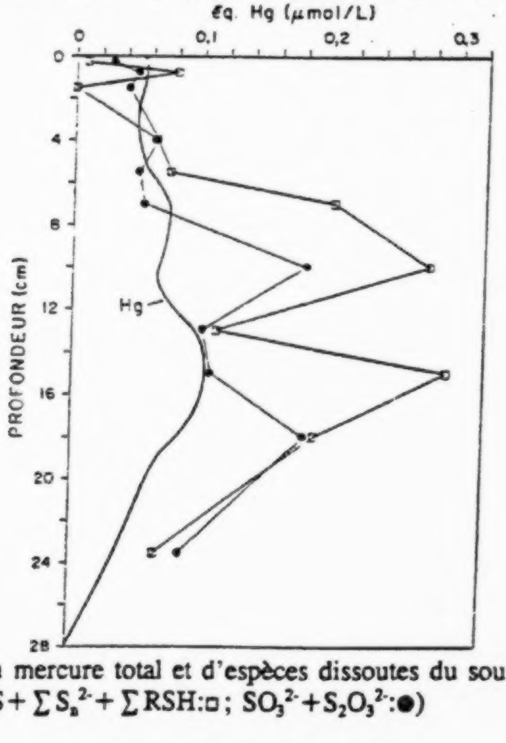

Figure 8. Distribution du mercure total et d'espèces dissoutes du soufre réduit dans la carotte SAG-30 ($\sum H_2S+\sum S_n^{2-}+\sum RSH$:□; $SO_3^{2-}+S_2O_3^{2-}$:●)

REMERCIEMENTS

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SEDIMENT/WATER DISTRIBUTION COEFFICIENTS AND SPECIATION OF CADMIUM IN FRESH WATERS.

R. Wagemann, M. Capel and R. Hesslein, Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba.

Sediment/water partition coefficients for cadmium in lake water were determined as a function of pH using ^{109}Cd, for three surficial bottom sediments and suspended matter from a Shield lake (L.382) in the Experimental Lakes Area of northwest Ontario. All the coefficients were found to be strongly pH dependent at $pH > 6.5$. In the pH range $6.5 \leq pH \leq 8$ the partition coefficient for suspended matter was much greater, ranging from 109×10^{-3} to 1579×10^{-3} (L mg^{-1}) than the bottom sediment coefficients ranging from 0.6×10^{-3} to 130×10^{-3} (L mg^{-1}). The lake was subsequently the site of a cadmium impact study on biota and received ^{109}Cd and cold cadmium which was maintained at approximately 100 ng/L in the lake. This was utilized to measure the fraction of cadmium adsorbed on suspended matter in the lake as a function of depth, and the results were compared with the values obtained by calculation. Speciation calculations were performed using the computer program MACS80. The computed fraction of the total cadmium in the water column associated with suspended matter at different depths in the lake agreed well with the measured fraction down to 7 m. In deeper water which was more acidic the measured fraction exceeded the computed fraction. Since hypolimnetic water was supersaturated with CO_2, some of the CO_2 may have escaped prior to counting causing an increase in pH and a shift in the equilibrium towards increased adsorption on suspended matter in the sample. This could explain the higher measured fraction of adsorbed cadmium in the more acidic, hypolimnetic water relative to the computed fraction. The association of cadmium with suspended and dissolved organic matter was strongly pH dependent. Under aerobic conditions at $pH > 6$, essentially > 90 % of cadmium in the water column was associated with suspended and dissolved organic matter, while at $pH < 6$, > 90 % cadmium was present as "free" cadmium. Under basic conditions, changes in the concentration of suspended and dissolved organic matter had a pronounced effect on the relative amount of cadmium associated with each of these constituents, but concentration changes in one or the other constituent had only a small effect on the fraction of "free" cadmium. The possible control of cadmium in this lake by such solids as $CdCO_3$, $Cd(OH)_2$, $Cd_3(PO_4)_2$ and CdS was also investigated by speciation calculations. The hydroxide and phosphate would allow relatively high equilibrium concentrations of cadmium in the water because of the low phosphate concentration and the predominantly acidic nature of this lake. These solids would therefore not be of any consequence in the control of cadmium in this lake and most oligotrophic Shield lakes. The water of L.382 was quite soft (1-2 mg/L TDIC, 2-3 mg/L Ca) and therefore high cadmium concentrations would also be allowed (tens of µg/L) by cadmium carbonate even at basic pH. Cadmium carbonate would therefore not control the cadmium concentration in this lake and most other Shield lakes with similar water quality characteristics. Under anaerobic conditions (in the winter) some of the lakes in this area are known to produce hydrogen sulfide. Whether or not L.382 also generates hydrogen sulfide in the winter is not known at this time. Speciation calculations indicated that under anaerobic conditions in the presence of even low concentrations of dissolved sulfide (5 µg/L) most cadmium would form CdS and eventually be removed from the water column, and any cadmium remaining in solution (in the ng/L range) would be in the form of sulfhydryl cadmium species, $Cd(HS)^+$ and $Cd(HS)_2$. Only insignificant amounts of the original cadmium in the water would remain associated with organic and suspended matter under these conditions.

SOLUBILITY ENHANCEMENT OF FENVALERATE BY ISOLATED DOC LAKEWATER FRACTIONS.

B.K. Burnison, Environment Canada, Rivers Research Branch, National Water Research Institute, Burlington, Ontario.

DOC fractions were isolated from the surface waters of six Ontario lakes using tangential flow ultrafiltration. The fractions were freeze-dried and analyzed for carbohydrate, protein, carbon, nitrogen, and iron. The molecular weight of the fractions obtained were verified by size exclusion chromatography. The ability of these fractions to enhance the solubility of ^{14}C-fenvalerate, a pyrethroid insecticide, was measured using the generator column technique. The solubility of fenvalerate in pure water was determined to be 2.8 µg/L. The high molecular weight DOC fractions (>30,000) bound more fenvalerate than the low molecular weight fraction (1,000->10,000). Log K_{doc} values for the > 30K fractions ranged from 4.89 to 5.99 and > 1K fractions ranged from 3.89 to 4.97. The value for commercial humic acid was 5.59.

RESUSPENSION OF PARTICLE BOUND CONTAMINANTS IN THE GREAT LAKES.

M.N. Charlton and M.E. Fox, Environment Canada, Lakes Research Branch, National Water Research Institute, Burlington, Ontario.

The resuspension of particles is a natural process which results in sediment focussing in lakes. Studies with sediment traps have been conducted in the Bay of Quinte, St. Clair River, Lake St. Clair, Lake Erie, Lake Ontario, and St. Lawrence River. The resuspending physical forces are sufficient to move sand grains nearshore but these are attenuated with depth so that only new flocculent material is resuspended at the bottom of Lake Ontario. In shallow situations the contamination of trapped material scarcely differs from surficial sediments. In deep water, a thin layer of the most recent depositing material is rapidly recirculated into the water column. The process is most intense in the winter resulting in enhanced decomposition as well as seasonal and spatial variability in particulate contaminants. Potential for repartitioning may occur during particularly strong resuspension events.

COMPLEXATION OF RADIONUCLIDES AND TRACE METALS BY DISSOLVED ORGANIC CARBON.

R.J. Cornett, L.A. Chant and G.M. Milton, Environmental Research Branch, AECL Research, Chalk River Laboratories, Chalk River, Ontario.

We tested the hypothesis that the rate of trace metal and radionuclide transport from water to sediments is inversely proportional to humic acid concentration by manipulating the concentration of humic acids (HA) in experimental enclosures located in a small lake. Following the manipulation of the HA concentration, radioisotopes of Na, Mn, Co, Fe, Ni, Zn, Hg, Se, Tc, Sn, I, Ba, Cs, Ce, Pb, and Ra were injected as soluble salts into the water. During the next 14 days, tracer concentrations in the water declined exponentially as the tracers were transported rapidly (-0.05-12% d^{-1}) from the water to the bottom sediments. The loss rates of the tracers were up to two times lower in the enclosures with higher concentrations of humic acids. These decreased loss rates of individual tracers correlate with an increase in the fraction of the tracer found in an anionic form. Tracer concentrations can be simulated effectively by a mass balance model that includes a pool of tracer in an anionic form that is not particle reactive.

ABIOTIC FACTORS THAT INFLUENCE TRACE METAL DISTRIBUTION AMONG SEDIMENT AND SOLUTION PHASES IN A SMALL URBANIZED RIVER.

L.A. Warren and A.P. Zimmerman, Department of Zoology, University of Toronto, Toronto, Ontario.

The distribution of trace metals between solution and sediment phases affects their transport, bioavailability and toxicity. We are assessing the geochemical partitioning of Cd, Cu, Pb and Zn with suspended particulate matter (SPM) in the Don River. The Don is a small, heavily urbanized system which runs through Metropolitan Toronto before emptying into Lake Ontario. Due to the variety and number of discharge inputs to the river, the Don provides a field opportunity to assess the potential abiotic factors as dissolved Cl^- concentration in controlling trace metal partitioning between sediment and solution phases; as well as assessment of potential competition between organic and oxide sediment components for trace metals. Preliminary results indicate that while absolute concentration and sediment geochemical partitioning vary substantially among the four trace metals, significant relationships occur between dissolved Cl^- and dissolved to sorbed metal ratios for Cd, Cu and Zn, in a manner consistent with ionic potential differences among the three trace metals. Regression analyses indicate that POC appears to enhance trace metal scavenging by iron oxides for Cd, Zn and Pb, as reducible metal to reducible iron ratios are positively related to POC concentrations.

SESSION 1B

BIOTIC FACTORS WHICH INFLUENCE CONTAMINANT TRANSFER/FACTEURS BIOTIQUES QUI INFLUENCENT LE TRANSFERT DES CONTAMINANTS

CHAIRPERSONS/PRÉSIDENTES
Landis Hare and Norman Yan

BIOCONCENTRATION ET DISTRIBUTION À TRÉS COURT TERME DE $^{203}HgCl_2$ ET $CH_3{}^{203}HgCl$ CHEZ L'ÉTOILE DE MER *Asterias rubens*.

C. Rouleau, E. Pelletier et H. Tjälve, Centre Océanographique de Rimouski, Rimouski, Québec, et Sveriges Lantbruksuniversitet, Uppsala, Sweden.

RÉSUMÉ

Nous avons étudié la bioconcentration et la distribution de $^{203}HgCl_2$ et $CH_3{}^{203}HgCl$ chez *Asterias rubens* à petite échelle temporelle. Deux groupes de 5 étoiles de mer ont été exposés à $^{203}HgCl_2$ et $CH_3{}^{203}HgCl$ respectivement, à une concentration de 200 ng $Hg{\cdot}g^{-1}$ (0,14 $\mu Ci{\cdot}L^{-1}$) dans l'eau de mer artificielle ($S = 35^{\circ}/_{\infty}$, $T = 11°C$). Trois étoiles par groupe ont été disséquées et la radioactivité de tous les tissus et organes a été mesurée à l'aide d'un compteur gamma. Les deux autres étoiles ont été utilisées pour la macroautoradiographie. Le contenu total en mercure est trois fois plus élevé pour le groupe exposé au méthylmercure, mais presque tout le mercure (94%) se retrouve sur la surface extérieure de l'animal. Seulement 77% du mercure se retrouve sur la surface extérieure pour le groupe exposé au mercure inorganique. Les autoradiogrammes révèlent une distribution hétérogène du mercure sur la carapace. Cette différence de la bioconcentration et de la distribution des différentes espèces de mercure chez *Asterias rubens* pourrait être due à la très grande affinité du méthylmercure pour les groupements sulfhydryles et à la faible concentration en ces mêmes groupements du liquide coelomique, lequel baigne les organes internes et est le principal moyen de transport interne. Le liquide coelomique pourrait être une obstacle au transfert du méthylmercure de la surface extérieure de l'animal vers les organes internes.

ABSTRACT

The bioconcentration and the distribution of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the starfish *Asterias rubens* was studied on a short temporal scale. Two groups of 5 starfish have been exposed 24 hours to $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ respectively, at a concentration of 200 ng $Hg{\cdot}L^{-1}$ (0.14 $\mu Ci{\cdot}L^{-1}$) in artificial sea water ($S = 35^{\circ}/_{\infty}$, $T = 11°C$). Three starfish per group were dissected and radioactivity of organs and tissues was determined by impulse counting. The other starfish were used for whole body autoradiography. Mercury body burden was three times higher for starfish exposed to $CH_3{}^{203}HgCl$, but most of the methylmercury (94%) was located on the outer surface while only 77% for the inorganic mercury was outside. Autoradiograms showed the heterogeneous distribution of mercury on the body wall. These differences in the distribution and bioconcentration of mercury in the starfish may be due to the very high affinity of methylmercury for sulfhydryl groups and the low concentration of these groups in the coelomic fluid surrounding internal organs which provides the main internal transport means. The coelomic fluid may act as a barrier to the transfer of methylmercury from the outer surface of the animal to the inner organs.

INTRODUCTION

Dans le cadre d'un programme de recherche sur le transport et les effets des composés organométalliques dans l'estuaire du St-Laurent, nous étudions plus particulièrement la cinétique de bioconcentration à très court terme de doses sous-létales de ces composés marqués radioactivement chez l'étoile de mer. L'utilisation de l'étoile de mer comme modèle biologique se justifie par sa large distribution dans l'estuaire du St-Laurent (Asterias vulgaris et Lepasterias polaris sont les principales espèces), la facilité de sa capture et sa bonne acclimatation aux conditions de laboratoire. Des travaux antérieurs sur la bioconcentration et la distribution du mercure chez les organismes aquatiques ont démontré que le méthylmercure est généralement accumulé et distribué à l'ensemble de l'organisme plus rapidement que le mercure inorganique (Boudou et Ribeyre, 1983, 1984; Riisgard et Famme, 1986; Pelletier, 1986, 1988; Gottofrey and Tjälve, 1991). Ce comportement particulier du méthylmercure est dû à sa liposolubilité et à sa grande affinité pour les groupements sulfhydryles (Carty et Malone, 1979; Rabenstein, 1978). Cependant, très peu de publications existent sur la bioconcentration du mercure chez l'étoile de mer (Sørensen et Bjerregaard, 1991) et, à notre connaissance, il n'existe aucune donnée sur la bioconcentration et la distribution du mercure inorganique et du méthylmercure à petite échelle temporelle chez cet animal.

La macroautoradiographie (Ullberg, 1977) est une technique de visualisation de la distribution d'un composé marqué dans des sections fines d'un animal entier. En plus d'être un moyen de contrôle des données quantitatives obtenues par comptage gamma sur les organes et tissus disséqués, la macroautoradiographie permet d'étudier en détail la distribution d'un composé marqué dans des structures trop petites, trop diffuses ou trop fragiles pour être disséquées. Cette technique, surtout utilisée en toxicologie et en pharmacologie classiques en Suède, a été appliquée à l'étude de la bioconcentration des métaux lourds chez le poisson (Tjälve et Gottofrey, 1991) mais ne l'a jamais été à l'écotoxicologie marine.

Nous rapportons ici les résultats, obtenus par comptage gamma et macroautoradiographie, d'une expérience sur la bioconcentration et la distribution du mercure chez Asterias rubens exposé 24 heures à une concentration sous-létale de $^{203}HgCl_2$ et $CH_3{}^{203}HgCl$.

MATÉRIEL ET MÉTHODES

Le chlorure de mercure (II) radioactif (0,7 $mCi \cdot mg^{-1}$) a été acheté chez Amersham. Le chlorure de méthylmercure radioactif a été synthétisé selon la méthode de Toribara (1985). Les étoiles de mer ont été recueillies sur la côte ouest de la Suède, près de Göteborg, et transportées rapidement au laboratoire à Uppsala où elles ont été acclimatées pendant trois jours dans des aquariums de verre contenant 40 L d'eau de mer artificielle (Instant Ocean®) à 35 $^{o}/_{oo}$, à une température de 11°C. Deux groupes de 5 étoiles de mer ont été exposés 24 heures à $^{203}HgCl_2$ et $CH_3{}^{203}HgCl$ respectivement, à une concentration de 200 ng $Hg \cdot L^{-1}$ (0,14 $\mu Ci \cdot L^{-1}$). Trois étoiles par groupe ont été disséquées et la radioactivité de l'estomac cardiaque, de l'estomac pylorique, du madréporite, des gonades, des caeca pyloriques, du liquide coelomique, de la bouche, des podia et de la carapace a été mesurée à l'aide d'un spectromètre gamma. Toutes les données ont été corrigées pour le bruit de fond et la désintégration de l'isotope et converties en ng de mercure. Les deux autres étoiles de chaque groupe ont été utilisées pour la macroautoradiographie selon la méthode décrite par Ullberg (1977). Elles ont été incluses entières dans un gel de carboxyméthylcellulose et congelées rapidement dans un mélange d'hexane et de glace sèche. Des sections de 40 μm d'épaisseur ont été faites dans un cryostat et lyophilisées à -20°C pendant 48 heures. Les sections sèches ont ensuite été mises en contact avec un film pour rayon-X régulier pendant 3 mois à -20°C.

RÉSULTATS ET DISCUSSION

Asterias rubens est une étoile de mer à 5 bras de la famille des Asteridae (fig. 1). La bouche, située au centre de la face ventrale, est reliée à un grand estomac, l'estomac cardiaque, lui-même relié à l'estomac pylorique, plus petit. A ce dernier sont branchés 5 paires de caeca pyloriques, une dans chaque bras, et un court intestin menant au caecum rectal. Celui-ci se termine par un anus débouchant sur la face supérieure de l'étoile de mer. On retrouve également dans chaque bras une paire de gonades. Toute la cavité interne de l'étoile de mer est remplie par le liquide coelomique qui constitue le principal moyen de transport interne. Sur toute la longueur de la face inférieure des bras, on retrouve le sillon ambulacral auquel sont rattachés les podia assurant la locomotion de l'étoile. Les podia sont reliés les uns aux autres par le canal radial. Chaque canal radial rejoint un canal annulaire situé autour de la bouche, lui-même relié au madréporite. Cette ensemble, le système vasculaire aqueux, constitue en quelque sorte le système hydraulique permettant à l'étoile de mer de se mouvoir. La carapace est constituée d'ossicules calcaires assemblés en réseau et dont la cohésion est assurée par du tissu conjonctif. On y retrouve entre autres les papules qui constituent, avec les podia, la principale surface d'échange avec le milieu aqueux (Barnes, 1974).

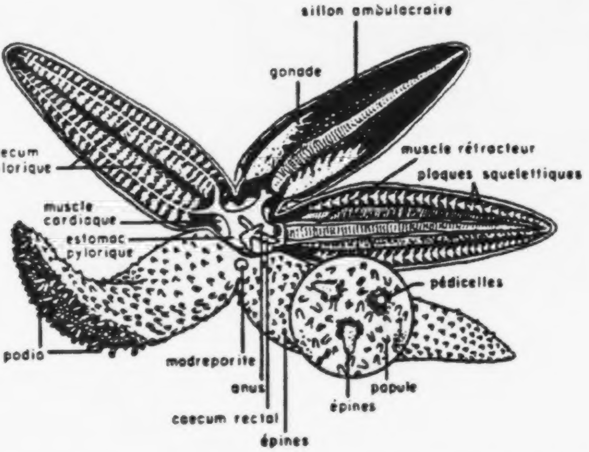

Fig. 1: Anatomie générale de l'étoile de mer (Asteridae). Adapté de Barnes (1974).

Après 24 heures d'exposition le contenu en mercure total des étoiles exposées au méthylmercure est trois fois plus élevé que pour celles exposées au mercure inorganique: 7,40 ± 2,34 ng $Hg \cdot g^{-1}$ vs 2,37 ± 0,41 ng $Hg \cdot g^{-1}$ ($p < 0,05$). Ceci donne un facteur de bioaccumulation de 37 pour le méthylmercure et de 12 pour le mercure inorganique. Même pour une très courte exposition, le méthylmercure est donc accumulé plus rapidement que le mercure inorganique chez Asterias rubens. La distribution du mercure dans l'animal est cependant différente pour les deux groupes. Pour le groupe d'étoiles exposé à $^{203}HgCl_2$, la concentration en mercure total des organes internes tend à être plus élevée que pour l'autre groupe, bien que cette différence ne soit significative que pour le madréporite et les gonades (fig. 2). A remarquer que la concentration de mercure dans le madréporite, très élevée pour ce groupe, est sous la limite de détection pour le groupe exposé au méthylmercure. Le groupe exposé à $CH_3{}^{203}HgCl$ a une concentration plus élevée en mercure total dans les podia (2,4 X) et la carapace (4 X). Le pourcentage du contenu total en mercure des organes internes du groupe exposé au mercure inorganique est significativement plus élevée que pour l'autre groupe (fig. 3). La proportion du mercure dans les podia est aussi un peu plus élevée. Cependant, 60% du mercure se retrouve dans la carapace pour le groupe traité au méthylmercure. Ainsi, pour ce groupe, 94% du contenu total en mercure de l'animal se retrouve sur les surfaces extérieures (podia et carapace) alors que cette proportion tombe à 77% pour le groupe exposé au mercure inorganique. Il semble donc que malgré un gradient de concentration plus important entre les surfaces externes et l'intérieur de l'animal, le méthylmercure pénètre plus difficilement. Les autoradiogrammes confirment cette distribution (fig. 4 et 5). Ils montrent aussi que le mercure, quelque soit sa forme, est distribué inégalement sur la carapace; le mercure pourrait se concentrer préférentiellement sur la matrice organique de la carapace (papules, pédicelles). On constate également que du mercure se retrouve dans le système vasculaire aqueux de l'étoile de mer pour le groupe exposé au mercure inorganique (fig. 6).

Cette différence de la distribution du mercure pourrait être due à la très grande affinité du méthylmercure pour les groupements sulfhydryles et à la physiologie particulière de l'étoile de mer. Il a été démontré que le méthylmercure à une très grande affinité pour les groupements sulfhydryles et qu'il se distribue rapidement à cause de la cinétique d'échange rapide du méthylmercure entre les groupements sulfhydryles (Rabenstein, 1978). Dans le cas des poissons, la distribution de ce contaminant, qu'il entre par les branchies (contamination par l'eau) ou par le système digestif (contamination trophique), se fait par l'intermédiaire du sang. Le plasma sanguin est une solution riche en protéines et donc riche en groupements sulfhydryles auxquels le méthylmercure peut se lier et être ainsi distribué et mis en contact avec tous les tissus de l'animal contaminé. Chez l'étoile de mer, l'espace intérieur est rempli par le liquide coelomique dans lequel baigne tous les organes. Il a une composition ionique semblable à celle de l'eau de mer (Barnes, 1974) et ne contient que peu de protéines comparativement au plasma sanguin. Il est possible que le méthylmercure soit capturé dans la matrice organique des podia et de la carapace du fait de la pauvreté en groupements sulfhydryles du liquide coelomique. L'affinité du mercure inorganique pour les groupements sulfhydryles n'est pas négligeable mais elle est tout de même moins importante que celle du méthylmercure. Le mercure inorganique pourrait ainsi diffuser plus facilement vers le milieu intérieur de l'étoile de mer.

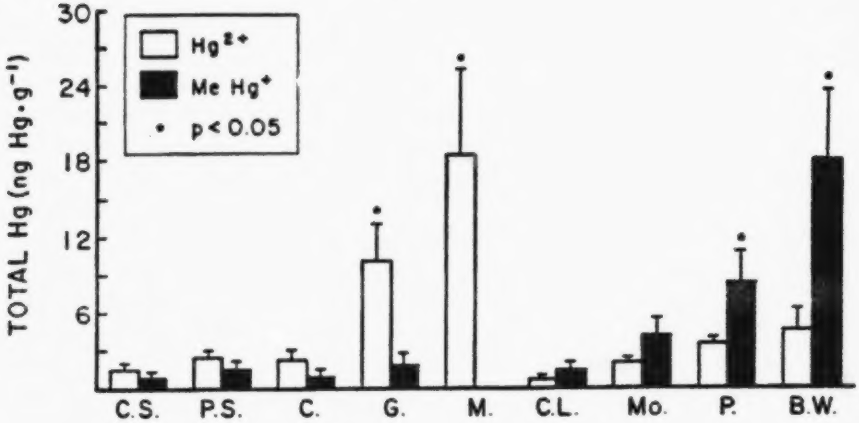

Fig. 2: Concentration en mercure total (X ± S.D., ng $Hg \cdot g^{-1}$) dans les divers tissus de Asterias rubens exposé 24 heures à $^{203}HgCl_2$ ou $CH_3{}^{203}HgCl$. C.S. = estomac cardiaque, P.S. = estomac pylorique, C. = caeca pyloriques, G. = gonades, M. = madréporite, C.L. = liquide coelomique, Mo. = bouche, P. = podia et B.W. = carapace.

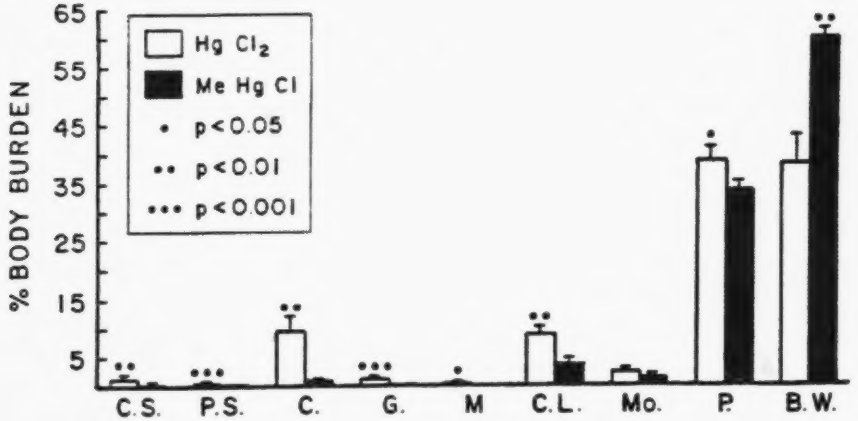

Fig. 3: Pourcentage du contenu total en mercure (X ± s.d.) des divers organes chez Asterias rubens exposé 24 heures à $^{203}HgCl_2$ ou $CH_3{}^{203}HgCl$. C.S. = estomac cardiaque, P.S. = estomac pylorique, C. = caeca pyloriques, G. = gonades, M. = madréporite, C.L. = liquide coelomique, Mo. = bouche, P. = podia et B.W. = carapace.

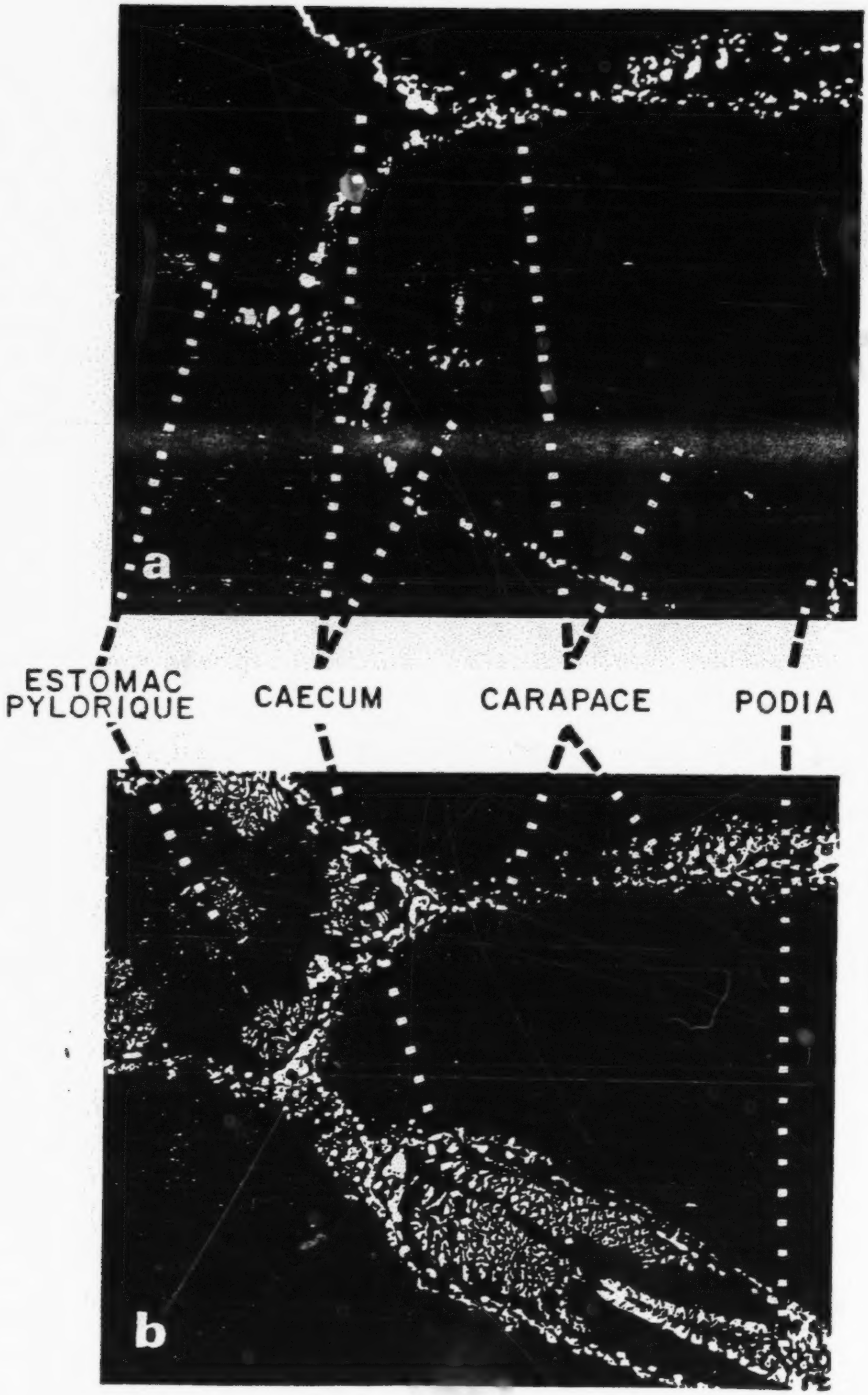
a
ESTOMAC
PYLORIQUE
CAECUM
CARAPACE
PODIA
b

Fig. 4: Détail d'un macroautoradiogramme d'une étoile de mer exposée au méthylmercure (200 ng Hg$\cdot$L^{-1}) pendant 24 heures (A). La section correspondante est illustrée en (B).

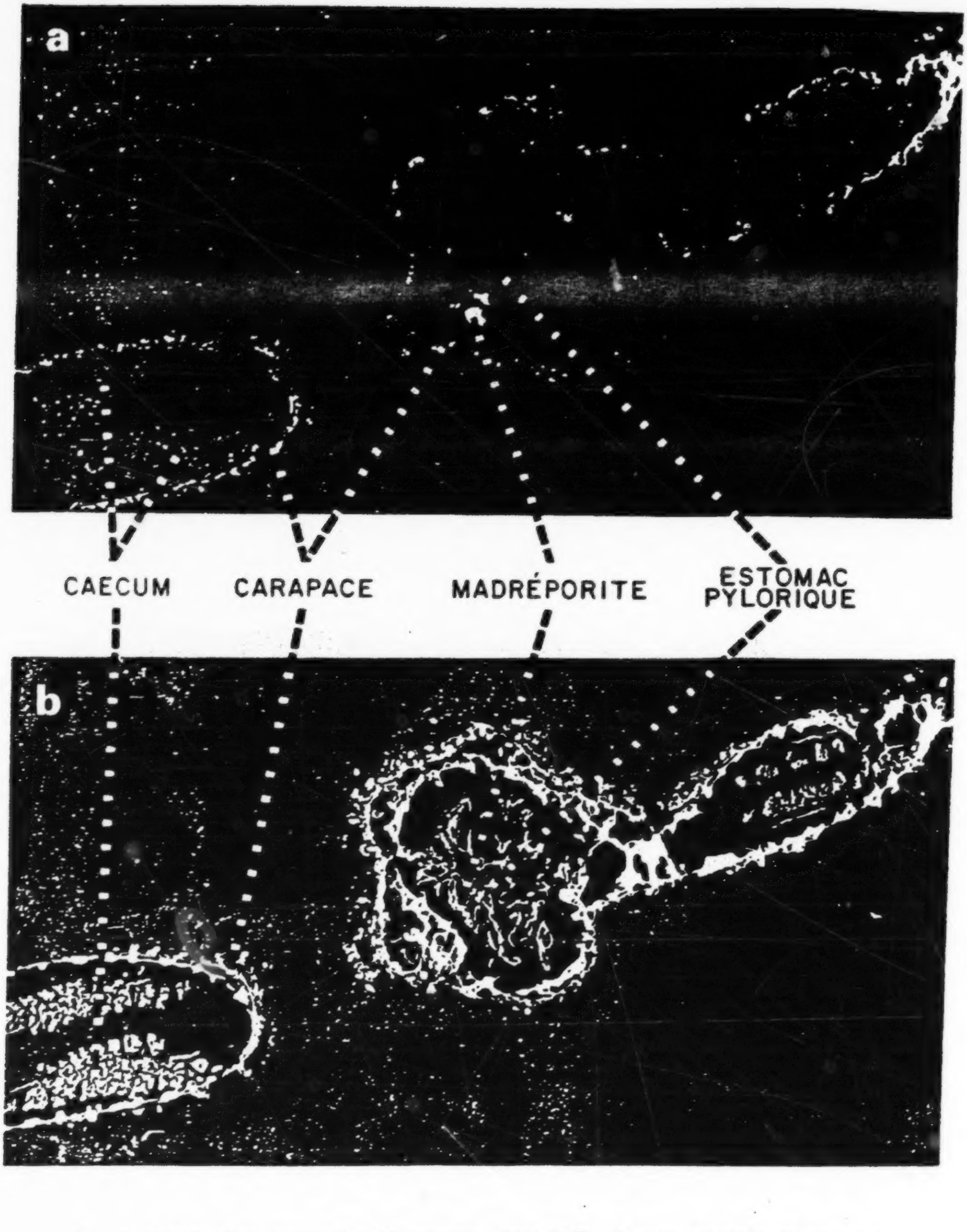
a
b
CAECUM
CARAPACE
MADRÉPORITE
ESTOMAC PYLORIQUE

Fig. 5: Détail d'un macroautoradiogramme d'une étoile de mer exposée au mercure inorganique (200 ng Hg·L^{-1}) pendant 24 heures (A). La section correspondante est illustrée en (B).

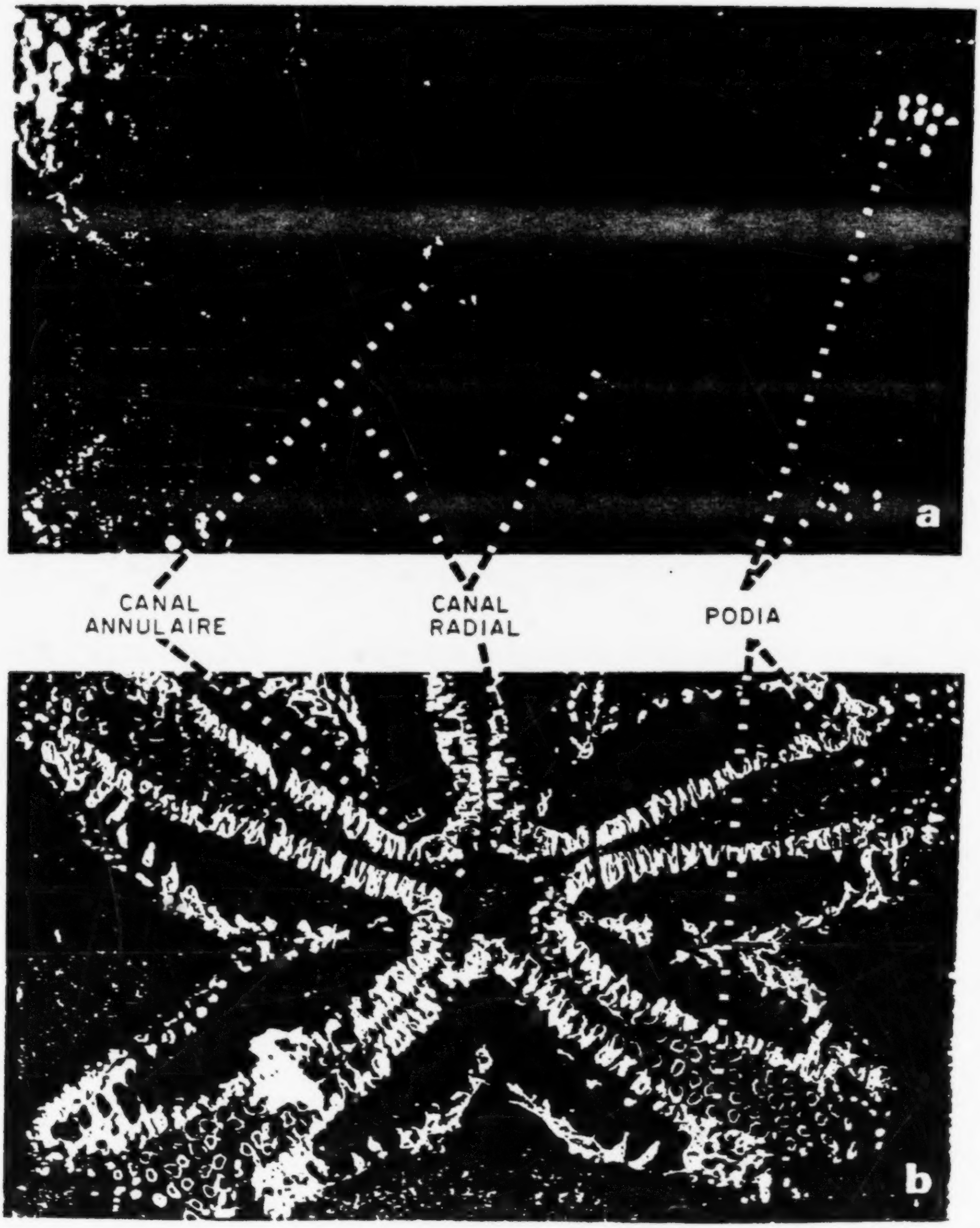
a
CANAL ANNULAIRE
CANAL RADIAL
PODIA
b

Fig. 6: Macroautoradiogramme d'une étoile de mer exposée au mercure inorganique (200 ng Hg·L^{-1}) pendant 24 heures. La section correspondante est illustrée en (B).

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Sørensen M. et P. Bjerregaard 1991. Interactive accumulation of mercury and selenium in the sea star Asterias rubens. Mar. Biol. 108: 269-276.

Tjälve H. et J. Gottofrey 1991. Effects of lipophilic complex formation on the uptake and distribution of some metals in fish. Pharmacology and Toxicology 60: 430-439.

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FATE OF MERCURY AND METHYLMERCURY IN STARFISH *Leptasterias polaris*: ORGAN DISTRIBUTION, FLUXES AND TRANSPORT MECHANISM.

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ABSTRACT

We studied modifications of short term distribution and transportation of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the starfish *Leptasterias polaris*. Mercaptans and amino acids were added to contaminated food (5ppm) or injected into the coelomic cavity. Two starfish per group were dissected and radioactivity of organs, tissues and seawater was determined by impulse counting. Mercury in food was distributes along the heamal system. Complexants modified distribution and transport to the pyloric caecum and the calcerous skeleton. Mercury excretion was also modified. Injected mercury in general cavity was absorbed variously by organs. Complexants modified mercury fixation on organ sites and mercury excretion was not significantly modified. It appeared that charge and size of complexes may have a role in diffusion trough membranes.

INTRODUCTION

The environmental chemistry and the toxicology of heavy metals in aquatic organisms have received an increasing interest in the last two decades. Among these metals, mercury is of particular interest being present under two chemical forms in the aquatic environment and being highly toxic to most living organisms (Carty and Malone, 1979). Alkylated forms of mercury show a great affinity to sulphydryl groups of proteins and are responsible for some severe neuro-intoxications (Rabenstein, 1978). Some laboratory works have established the bioaccumulation of both mercury chemical forms in marine organisms. Early studies by Penthreath (1976 a,b,c) on the american plaise *Pleuronectes platessa* evidenced the bioconcentration of inorganic mercury from water directly into skin and gills whereas methylmercury passed through biological membranes and accumulated in the fish muscle. The uptake of inorganic Hg from food showed bioaccumulation in gills and digestive organs while methylmercury accumulated mainly in intestine and liver. Using *Salmo gairderi* in bioassays, Boudou and Ribeyre (1984) demonstrated that the higher retention capability of methylmercury over inorganic mercury was independent from the uptake route.

In previous works (Pelletier and Larocque, 1989), the bioaccumulation of methylmercury in starfish *Leptasterias polaris* feeding contaminated mussels was reported. An auto-epuration process was postulated after four weeks of continuous uptake; methylmercury in digestive organs decreased even if contaminated food was still provided. This presentation gives results on the uptake and transportation mechanisms of inorganic mercury and methylmercury in starfish *Leptasterias polaris*. Experiments were conducted to determine the effects of various chemical chelatans and mercaptans added to food or injected into the starfish on the adsorption, the transport and the elimination of both chemical forms of mercury.

MATERIAL AND METHODS

Bioessai procedure:

Labelled mercury ($^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$) solutions were added to mercury contaminated mussel homogenate or seawater solution, as tracer.

A-Ingested from food: Starfish were force-feeded according to a procedure developped by Oudejans and Rutten (1982). Chemicals were added to the homogenate, immediatly prior the feeding. Briefly, contaminated mussel homogenate (5ppm Hg) was injected into the stomach by inserting a rounded stell needle in the mouth of the animal (fig 1). The following chemical complexant were used: alanine (ala), cysteine (cys), methyl-S-cyteine (Me-S-cys), glutathione (GSH), 2-mercaptosuccinic acid (succ), 2,3-dimercaptosuccinic acid (dimerc), mercaptoethanol (merOH) and EDTA.

B-Injected solution: Mercury solution (0,5 mL of a 10ppm Hg solution) was injected in the calcerous skeleton in the last third of an arm on the ventral side of the animal (fig 1). Complexant solutions, made in seawater, were injected in the opposite arm, immediatly after mercury injection and the animal was rotated to allow mixing of injected solutions in the starfish. Complexants were the same as those used in food experiment.

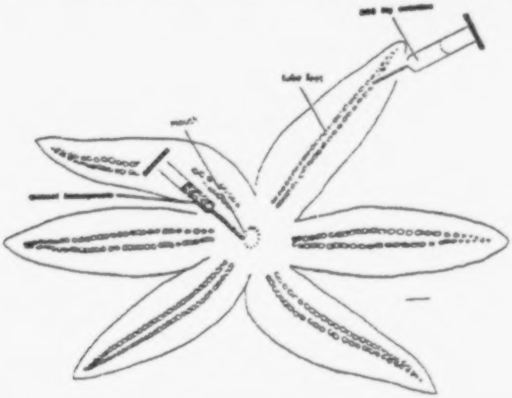


Figure 1 Contamination procedure

Once the animal was contaminated, it was placed into a glass beaker, containing 3L of fresh seawater. Air bubbling insured the mixing of seawater and temperature was kept constant (6^0 C). During the 24-hour exposition period, seawater was sampled (0, 0.5, 1, 2, 4, 6, 8 and 24 h) for mercury analysis.

After the 24 hour period, the animal was sacrified; the coelomic fluid was collected by cutting the end of one arm and suspending the starfish by the opposite arm. The liquid was weighted ans subsampled for ^{203}Hg gamma counting. The entire cardiac and pyloric stomachs, gonads, the pyloric caecum were dissected, while subsamples of calcerous skeleton were taken from each arm. All organ samples were weighted and counted for ^{203}Hg.

Mercury analysis

A-Mercury in tissues: Both $^{203}Hg^+$ and CH_3Hg^+ were analyzed by gamma counting on a Clinigamma LKB counter. Gamma emission of ^{203}Hg has an energy of 279,19 KeV and counting time was 5 minutes for each sample.

B-Mercury extracted from seawater: Inorganic mercury was recovered from seawater by dry-freezing. Precipitated salts were quantitatively resolubilized in 3mL of HCl (3N), transfered in a tube and counted for 203 mercury. Methylmercury was extracted according to Cappon and Smith (1977). Methylmercury was first complexed by urea, while $CuSO_4$ was added to aqueous phase in order to prevent complexes formation by sulphydryls bearing compounds. The aqueous phase was then acidified (HCl 5N) and twice extracted with benzene. The benzene phases were combined and counted for 203 mercury.

RESULTS

The asteroid *Leptasterias polaris* is a 6 armed star widely distributed in the St-Lawrence estuary and along the eastern canadian coast. Its physiology is similar to *Asterias rubens;* digestive structures in this large asteroidae family correspond to the classical scheme showed by Barnes (1974) (Fig.2). The mouth opens in the mid-ventral part of the body and leads directly into the cardiac stomach, wich is connected with the pyloric stomach. From the stomach rise pairs of branched dead-end pyloric caecum, each pair being located in the cavity of one arm. A short intestine gives rise to the rectal caeca linked to a small eccentric anus on the arboral part of the star (Jangoux 1982).

Internal organs, the pyloric caecum and gonads are suspended in the coelomic fluid that has a composition almost identical to external seawater (Barnes 1974).

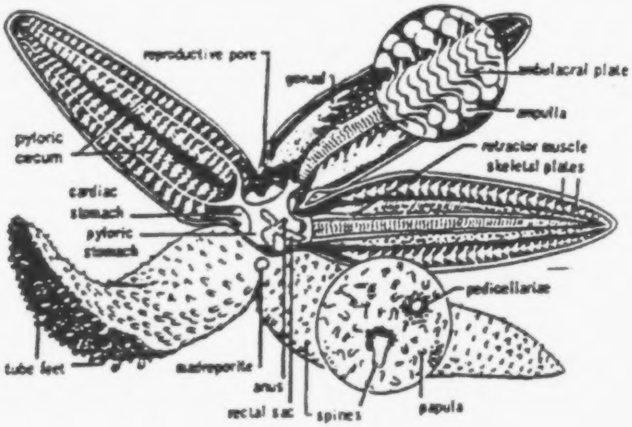

reproductive pore
gonad
ambulacral plate
ampulla
pyloric cecum
retractor muscle
skeletal plates
cardiac stomach
pyloric stomach
pedicellariae
tube feet
madreporite
anus
rectal sac
spines
papula

Figure 2 Starfish anatomy

1-Organ distribution of inorganic ^{203}Hg taken from food

Three different organ distributions of inorganic mercury in various tissues of *L. polaris* were observed when starfish fed contaminated mussel homogenates (Fig.3). Each bar graph gives the resulting organ distribution (in % of the retained dose) 24 h after the ingestion of contaminated food and a function of the complexant added to the food. The retained dose is calculated by adding all measured mercury quantities in the entiere starfish and expressing the amount from each organ as a percentage of the total found.

Mercury ingested in absence of any complexant in the food was accumulated mainly in pyloric caecum (83 % of the dose) and in calcerous skeleton (10 %), as shown in Fig.3a. Four complexants induced little changes from the mercury alone: glutathione (GSH), alanine (ala), cysteine (cys) and EDTA.

A clearly different organ distribution was observed when methyl-S-cysteine (Me-S-cys) and mercaptoethanol (merOH) were added to homogenates containing inorganic mercury (Fig.3b). An higher retention in stomach (up to 25%) and calcerous skeleton (36 ±3%) was observed while the retained dose in pyloric cecum lowered to 33% ±8% (compared to 83% for mercury alone).

A third distribution pattern was found when 2-mercaptosuccinic acid (succ) or 2,3-dimercaptosuccinic acid (dimerc) was added to the homogenate (Fig.3c). Mercury accumulated mainly in calcerous skeleton (>58% of retained dose compared to 10% for mercury alone) while pyloric caecum adsorbed less then 20 ±5% (compared to 83% alone) and almost nothing left in the stomach. These two complexants highly promoted transport of inorganic mercury in food from the pyloric caecum to the calcerous skeleton.

2-Organ distribution of $CH_3{}^{203}Hg$ taken from food

Three different distributions of methylmercury in various tissues of *L. polaris* feeding contaminated mussel homogenates are shown on Fig.4.

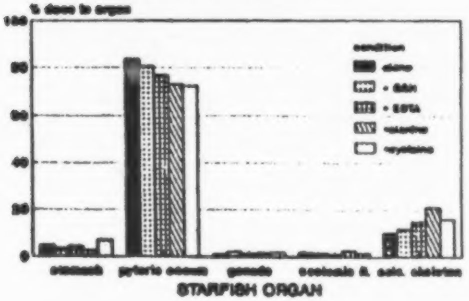
Methylmercury alone in food was mainly accumulated in pyloric caecum (60% of the retained dose) and in calcerous skeleton (28%) (Fig.4a); stomach retained 12% while gonads and coelomic fluid accumulated less than 1% of the dose. Five complexants (ala, Me-S-cys, succ, dimerc and GSH) did not modify that distribution.

The second Hg distribution pattern was observed when complexation occured with EDTA (Fig.4b). In that case, mercury was distributed almost evenly between stomach, pyloric caecum and calcerous skeleton (34%, 31% and 33% respectively).

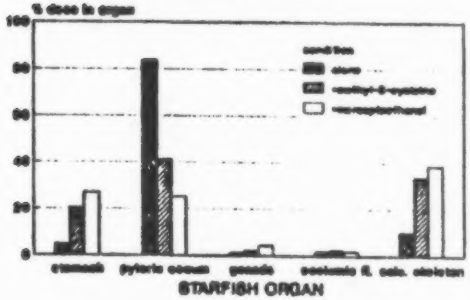
Finally, a third pattern was found in presence of cys and merOH (Fig.4c). With these two complexants, calcerous skeleton became the major site of methylmercury accumulation (55% of the dose compared to 28% alone), retained dose in pyloric caecum dropped from 60% MeHg alone to 23%, while retained dose in stomach remained relatively high (to 12%). The retained dose in gonads went up to 7%. These two complexants promoted transfert of methylmercury from the pyloric caecum to the calcerous skeleton and to some extend to stomach.

Figure 3: 203HgCl2 in food

organ distribution 1



organ distribution 2



organ distribution 3

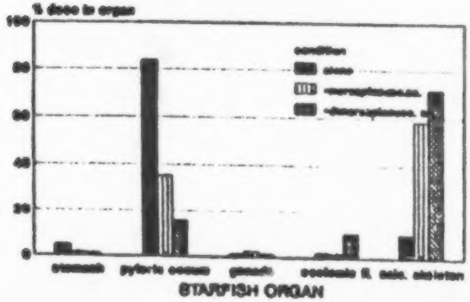
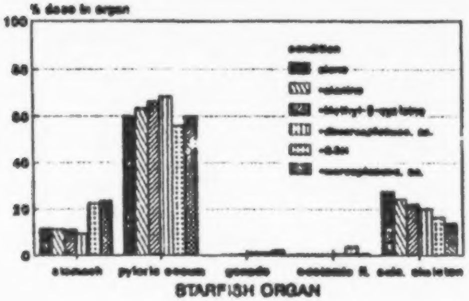
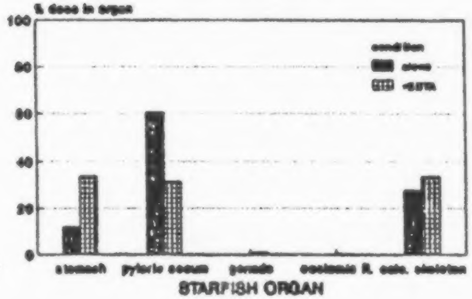


Figure 4:CH3 203Hg in food

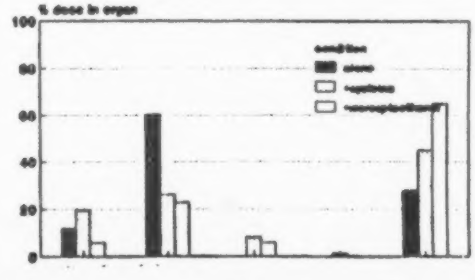
organ distribution 1



organ distribution 2



organ distribution 3



3-Organs distribution of inorganic ^{203}Hg injected in coelomic fluid

Three distributions of inorganic mercury were observed in various tissues of *L. polaris* receiving injected Hg solution in arms (Fig.5).

Inorganic mercury injected alone was absorbed mainly by calcerous skeleton (50%) and pyloric cecum (25%) (Fig.5a). The dose remaining in coelomic fluid and gonads were 12% and 11% respectively. A very small portion of the dose (2%) migrated to the stomach. Five complexants (ala, Me-S-cys, cys, succ and EDTA) injected in the coelomic cavity after the mercury injection did not modify significantly this distribution.

A second organ distribution was observed when GSH or dimerc was injected subsequently to mercury (Fig.5b). Mercury was still mainly absorbed by calcerous skeleton (45% of the dose) but absorbed dose by pyloric cecum decreased to 15% while dose trapped in the coelomic fluid increased to 31% (compared to 12% alone).

Finally, a peculiar third organ distribution was found when the complexant was merOH, (Fig.5c). In that case, mercury was mainly measured in pyloric cecum (45% of the dose) while calcerous skeleton absorbed 31% of the total dose compared to 50% for mercury alone. It should be noted that mercury remaining in coelomic fluid was very low (5%) in that particular case.

4-Organs distribution of $CH_3{}^{203}Hg$ from injected solution

As observed in previous cases three methylmercury distributions were observed in various tissues of *L. polaris* receiving methylmercury solution in the coelomic fluid (Fig.6).

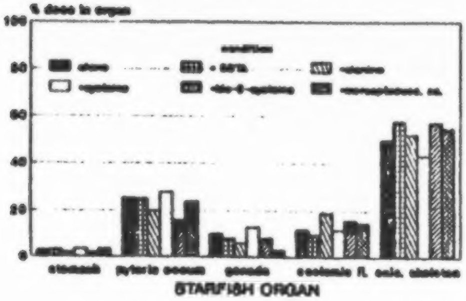
Methylmercury injected alone was absorbed mainly by calcerous skeleton and pyloric caecum (33% in each of them) (Fig.6a). The absorption in gonads was 14% of dose and the dose remaining in coelomic fluid was 17%. There was a small migration to the stomach (3%). Three complexants did not modify very much that distribution: cys, succ and dimerc.

A second organ distribution was observed when ala, Me-S-cys or EDTA were injected subsequently to methylmercury (Fig.6b). In that pattern, methylmercury was mainly aborbed in calcerous skeleton (37%) whereas absorbtion by pyloric caecum was lowered (18% compared to 33% alone). The gonads retention increased to 15%. The dose remaining in coelomic fluid for these three chemicals was higher than any other complexant and averaged 26% (compared to 17% alone).

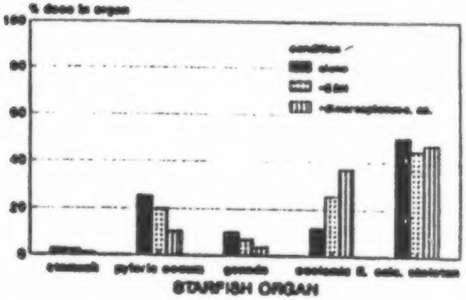
A third organ distribution was observed when GSH or merOH were injected subsequently to methylmercury. As shown on Figure 6c, absorbtion on calcerous skeleton was high (56 ±8%) compared to 33% for methylmercury alone, absorbtion in pyloric caecum and gonads did not vary (32 ±6% and 7 ±3% respectively). However, the portion of the dose remaining in coelomic fluid was strongly lowered to less than 3%. These two complexants seems to promote a retention by calcerous skeleton.

Figure 5:203HgCl2 injected

organ distribution 1



organ distribution 2



organ distribution 3

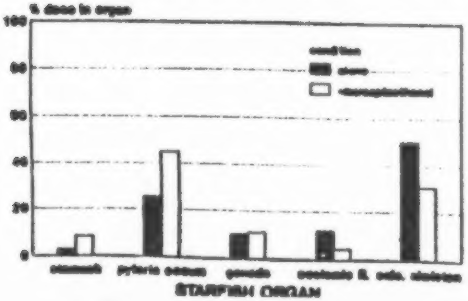
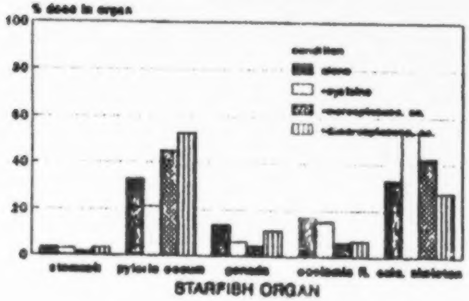
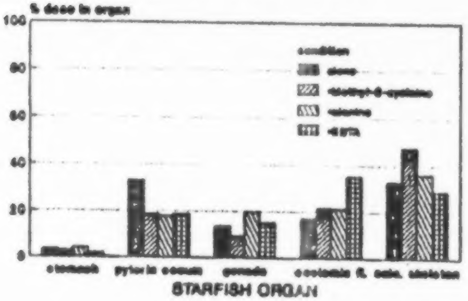


Figure 6:CH3 203Hg injected

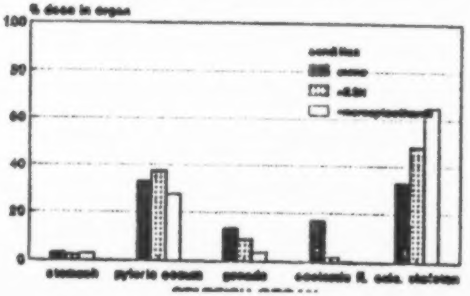
organ distribution 1



organ distribution 2



organ distribution 3



DISCUSSION

The utilisation of complexants to modify accumulation of heavy metal by marine organisms is relatively well studied (Georges and Coombs, (1977), Ahsanullah and Florence, (1984), Nugegoda and Rainbow, (1988), Guttierez-Galindo, (1981)). In these works complexation of metal occured in seawater in order to study biodisponibility of metal on marine organism. To our knowledge, there is no studies on complexation in food or in injected solution to marine organisms.

1-Mercury from ingested food

Whenever inorganic mercury or methylmercury were present in food, their short-term distribution and accumulation occured in same organs. Bioaccumulation occured in three of the five studied organs: stomach (in contact with the contaminated food), pyloric caecum and calcerous skeleton. The coelomic fluid and gonads did not significantly accumulate mercury on a short-term 24h period (Fig3 and 4). Organs accumulating most of the mercury are linked to the hemal system, as described by Cuénot (1948), Barnes (1974) et Fergusson (1982).

Briefly, the hemal system (Fig.7) consists of small fluid-filled channels wich are surrounded by separate extention of the coelom called sinuses. The main hemal channel consits of an oral hemal ring located beyong the periphery of the perisome. From this ring, a radial hemal sinus extend into each arm and lie beneath the oral surface along the midline of the ambulacral groove. From the oral hemal ring, a channel ascends along the stone canal. In addition to the ascending channel from the oral hemal ring, the axial gland also receives small channels from the pyloric caecum and the walls of the cardiac stomach. Branches to the gonads issue from the aboral hemal disc giving rise to an elaborated hemal sinus.

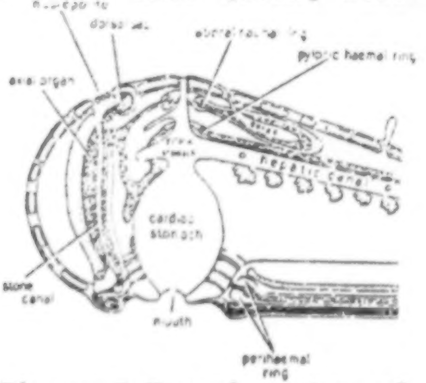


Figure 7 Haemal system of starfish

Observed distributions of mercury from food are consistent with the model where mercury is translocated to the organs via the hemal system which links together the digestive system, the calcerous skeleton and the gonads. Calcerous skeleton and pyloric caecum are the largest organs of *L. polaris* (over 80% of total body weight), wich explain why over 85% of the mercury ($HgCl_2$ or CH_3Hg) was found in those organs.

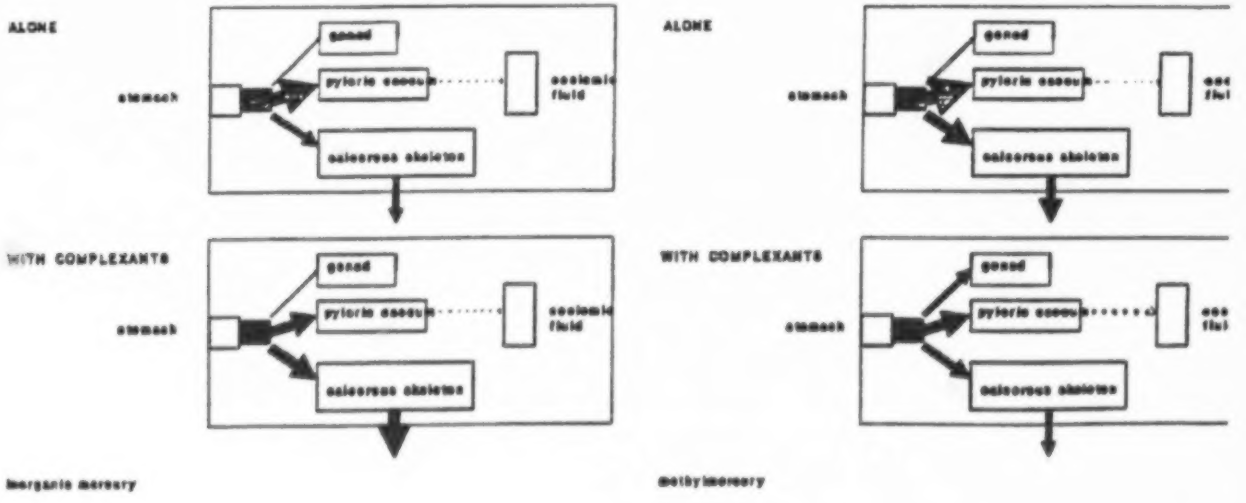
Mercury might be bound to glycoproteins like those found in starfish gonads (Broertjes, 1984). These glycoproteins were also detected in the hemal system, in the aboral skin, in coelomocytes contained in hemal sinuses and in mucous lining several internal organs (Beijnink and coll., 1984a). It was suggested that the glycoproteins were not exclusively used for vitellogenesis but they may serve as building material for other parts of the body. They could also serve as a complex form of nutrient translocation or heavy metal transportation since proteins bear sulfydryl groups for which mercury and other metals have a high affinity.

Count per minute can be transposed to mercury quantities by interpolation on a calibration curve. If we standardize food quantity ingested to 1g and animal weight to 100g, we then have standard animal receiving a standard dose. The mercury quantity in a standard organ can be divided by elapsed time (24 hours), thus giving fluxes to organs.

Complexants behave very differently with inorganic mercury or methylmercury (fig. 8). Complexants diminished transport of inorganic mercury to pyloric caecum. The transport to calcerous skeleton was increased and consequently, mercury excretion was also enhanced. In any cases were transport to gonads or diffusion to coelomic fluid increased.

On the other hand, complexants increased methylmercury transport to the gonads and its diffusion in the coelomic fluid. Consequently, transport to calcerous skeleton and excretion of methylmercury were diminished.

Figure 8 Effect of complexants on mercury and methylmercury transport in *L. polaris* organs


ALONE
gonad
stomach
pyloric caecum
coelomic fluid
calcerous skeleton
WITH COMPLEXANTS
gonad
stomach
pyloric caecum
coelomic fluid
calcerous skeleton
inorganic mercury
ALONE
gonad
stomach
pyloric caecum
calcerous skeleton
WITH COMPLEXANTS
gonad
stomach
pyloric caecum
calcerous skeleton
methylmercury

2-Mercury from injected solutions

This relatively unusual procedure has been used by Ferguson (1964), Oudejans and Rutten (1982), Oudejans and coll. (1983) and Beijnink and coll. (1984b) to follow nutrient absorption from the general cavity to the different organs steeping in the coelomic fluid. This procedure differs from these experiments in the sense that complexation of mercury occurs *in vivo rather then* in vitro.

All organs present in the coelom (excluding the stomach) accumulated mercury from the coelomic fluid. Pyloric caecum and gonads are surrounded by coelomic fluid, wich creates an interface where absorption of mercury may occur. Calcerous skeleton posses an organic lining, wich gives rise to an equivalent surface for mercury fixation or absorption. Mercury could be fixed on sulfydryls groups of membrane proteins or on membrane phospholipids before diffusing trough the membrane. The composition of membrane could influence both fixation and diffusion processes of mercury.

It is now accepted that mercury does not cross membrane by active transport. Bienvenue and coll. (1984) demonstrated that $HgCl_2$ and CH_3HgCl were the chemical form under wich mercury and methylmercury diffused trough the membrane.

We can therefore assume that the accumulation and distribution results from this experiment reflect mercury diffusion accross three different membranes. The addition of complexants to the medium allow us to evaluate the affinity of membrane components (proteins and phospolipids) to complexes of mercury and methylmercury and the relative ability of these complexes to diffuse trough different membranes.

Gonads were the faster accumulating organ. They were followed by pyloric caecum and to a smaller extend by calcerous skeleton. This scheme was the same for the two chemical forms of mercury. This distribution was governed by solubility of both inorganic mercury or methylmercury in polar lipids contained in the different organs.

Assuming that complexation occurs in the coelomic fluid, the complex diffusion accross the membrane will depend on their size and affinity to the different membrane components, such as phospholipids and proteins.

If we transform count per minute in mercury quantity and work with a 100g standard animal, the diffusion rate can be assumed to be the mercury quantity found in a standard organ divided by elapsed time.

Diffusion of the complexes trough the different membranes differed in every cases. As an exemple, every complexants except cysteine, enhanced diffusion trough the gonad membrane. Complexation might increase organic character of Hg^{2+} ions, thus faciliting diffusion. With methylmercury, complexants diminished the flux to gonads with GSH being the noticeable exception. This tendency was strong with amino acids (cysteine, methyl-cysteine and alanine) and even stronger with mono and dimercaptosuccinic acids. It appears that carbonyl groups diminish methylmercury diffusion trough this membrane.

In conclusion, mercury added to food was transported by haemal system to the organs of *L. polaris*. Complexants enhanced transport of inorganic mercury to the calcerous skeleton where it was excreted in seawater. Complexants increased transport of methylmercury to the gonads and coelomic fluid, the excretion of the metal was lowered.

Mercury absorbtion by organs when injected in general cavity was governed by liposolubility in polar lipids. The presence of carboxyls and sulfydryls groups (present on complexants) modified the diffusion process of mercury trough the different membranes present in the general cavity.

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RELATIONSHIPS BETWEEN Cd^{2+} ACTIVITY CADMIUM BIOACCUMULATION AND SUBLETHAL TOXICITY IN THE POLYCHAETE Neanthes arenacedentata.

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SUMMARY

Concerns over the anthropogenic release of metals into coastal waters have resulted in the development of a variety of strategies for predicting the biological availability and subsequent toxicity of these inputs. Early chemical approaches to this problem were limited because the techniques for measuring metals in the marine environment did not accurately predict trace metal bioavailability (Sunda and Guillard, 1976). Subsequent studies indicated that bioavailability of many transition metals may be a function of the activity of the free metal ion in solution rather than the total concentration of metal in the water (Sunda and Guillard, 1976; Sunda). The relationships between free ion activities bioaccumulation and toxicity has only been established for a few species and metals. Moreover, free ion activities of transition metals are often difficult to measure in field samples and this approach has thus seen limited use. The problem of bioavailability can be addressed more directly by measuring tissue residue in organisms exposed in the field. However, it is often difficult to relate tissue residue data to the underlying chemistry or subsequent biological effects. This lack of correlation may reflect the capacity of the organism to regulate metal accumulation and to detoxify accumulated metals (Mason et al., 1988).

With these problems in mind, we have carried out a series of experiments which examine the relationship between free cadmium ion activity ($\{Cd^{2+}\}$) and Cd bioavailability, subcellular compartmentilization and sublethal toxicity in an isogenetic strain of the benthic polychaete Neanthes arenaceodentata (Reish, 1957; Reish and Richards 1966). An EDTA based metal chelate buffer system containing radioactive ^{109}Cd was used to control the speciation of cadmium in seawater. Groups of organisms were exposed to $\{Cd^{2+}\}$ ranging from 10^{-12} M to $10^{-6.5}$ M and the accumulation of ^{109}Cd was monitored over time. Double logarithmic plots of the body burden of metal (at the steady state) against external $\{Cd^{2+}\}$ show a linear dose-response relationship at low external concentration of $\{Cd^{2+}\}$ between 10^{-12} and 10^{-10} M with a proportionality constant of approximately one ($r^2=0.996$; slope 0.998). However, enhanced accumulation of Cd and a deviation away from proportionality was observed at external concentrations of $\{Cd^{2+}\}$ greater than 10^{-9} M due to a relative increase in the accumulation of Cd in the cytosol. This increase in cytosolic Cd was largely attributable to a relative increase in the concentration of Cd in two cytosolic ligand pools (Jenkins and Mason, 1988). At 10^{-9} M $\{Cd^{2+}\}$ there was elevated levels of Cd in the metallothionein (MT) pool which appeared to reflect induction of the protein. At concentrations greater than 10^{-8} M $\{Cd^{2+}\}$ there was reduced accumulation of Cd in MT relative to dose, but there was a dramatic increase in Cd in a very low molecular weight ligand pool with an apparent molecular weight of <5 kDa. The accumulation of Cd in this pool correlated with the onset of sublethal perturbations in growth and reproduction (Jenkins and Sanders, 1986; Jenkins and Mason, 1988).

These studies indicate that Cd accumulation is tightly coupled to free ion activities between 10^{-12} and 10^{-10} M. Calculations indicate that this range includes values which are typical for uncontaminated seawater which can vary from $10^{-11.4}$ and $10^{-10.4}$ M depending upon depth of sampling. Similar estimations indicate that the $\{Cd^{2+}\}$ in interstitial waters of heavily contaminated sediments may exceed $10^{-7.6}$ M. Although the data indicate that the animals exercise physiological control of accumulation at $\{Cd^{2+}\}$ greater than 10^{-9} M and no longer show proportional accumulation of metal, waters with elevated $\{Cd^{2+}\}$ could be readily identified by the accumulation of Cd in the VLMW ligand pool. This increased accumulation of Cd in the VLMW pool also reflects higher level biological effects including compromised growth and reproduction.

Based upon the studies outlined above, it would appear that N. arenaceodentata may serve as an excellent biological indicator of both the bioavailability and the toxicity of metals such as Cd in sediments. By calibrating the body burden of metal, subcellular metal distribution and physiological response (i.e. growth and reproductive potential) against external $\{Cd^{2+}\}$, we have identified a suite of measurable responses which have the potential to indirectly quantify low $\{Cd^{2+}\}$ seawater concentrations and register elevated levels of the metal which result in sublethal stress.

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ADJUSTING CONTAMINANT CONCENTRATIONS FOR FISH-SIZE COVARIATION.

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Ratios are frequently used to standardize contaminant data and thereby 'control' for otherwise uninteresting variation in fish size. Unfortunately, ratios may fail to remove the confounding influence of the denominator (or divisor) and hence, the widespread use of ratios is frequently criticized. Two philosophies have arisen from the debate surrounding the pros and cons of using ratios and related indices. One philosophy recognises that many of the requisite assumptions for the use of ratios are rarely met, and hence, advocates regression-based alternatives (e.g. the analysis of covariance or ANCOVA; Jackson et al. 1990). The second philosophy proposes that ratios are useful constructs that permit the evaluation of otherwise intractable hypotheses (e.g. lipid-corrected PCB concentration does not differ among sites; see Jackson and Somers [1991] for details).

The recent availability of fast microcomputers has rekindled interest in computationally demanding randomization tests that generate expecteddistributions for various statistics without the traditional assumptionsinherent with the use of statistical tables (e.g. see Edgington 1987; Manly 1991). Here, we demonstrate the use of randomization tests with the analysis of variance (ANOVA) and ANCOVA to explore differences in contaminant concentrations that covary with fish size. Using data on mercury and PCB concentrations from two populations of lake trout (Salvelinus namaycush) from Lake Superior, we contrast results of regression-based adjustments and ratios using standard statistical tables and randomizations.

Samples of dorsal-muscle fillets were collected from a population of nearshore lake trout and a deep-water population (commonly called siscowets or fats because of their 10-35% lipid content) and analysed for mercury and PCB concentrations in the late 1970's and mid-to-late 1980's as part of the Ontario sportfish contaminants monitoring program. Because these two contaminants tend to covary with length and lipid concentration, and because average length and percent lipid varied between populations and dates, contaminant concentrations were adjusted for length and/or lipid with bivariate regressions and ratios.

Using ANCOVAs, slopes of the contaminant concentrations relative to fish length differed significantly with respect to population and date (i.e. the two-way interaction) indicating that the regressions with size were not parallel. This finding suggests that the rate of change of contaminant concentration (i.e. uptake) with respect to fish size differsbetween populations over the two dates. When both length and lipid were included in a multiple ANCOVA, the four regressions (i.e. two populations by two dates) were parallel, but the intercepts differed (i.e. the adjusted concentrations of the more-recent samples from the nearshore lake trout populations were lower, whereas concentrations in siscowets were somewhat higher).

Comparisons based on lipid-corrected PCB (i.e. the ratio of PCB divided by lipid) or length-adjusted mercury indicated significant interactions in a two-way ANOVA (resembling the results from the multiple ANCOVA). However, none of the F values was significant for the lipid-corrected PCB data using randomization tests. This result indicated that differences in lipid concentrations paralleled differences in PCB concentrations, such that the ratio correction inadvertently reduced differences in PCBs to that attributable to random noise. In contrast, randomization tests of the length-adjusted mercury data provided F values with probabilities that were nearly identical to probabilities from standard statistical tables.

ANCOVAs with length as a covariate revealed significantly different slopes among the four regressions for lipid-adjusted PCBs and among the length-standardized mercury data. Again however, the randomization test indicated no significant differences among the regressions for lipid-corrected PCBs (i.e. differences in PCB concentrations wereeliminated with the ratio correction). In contrast, the four regressions using length-corrected mercury exhibited significantly different slopes with both the randomization test and standard statistical tables. As a result, the randomization test indicated that the lipid correction compromised the analysis of the PCB data, but the length correction did not bias the analysis of the mercury data.

Differences in the results for corrected PCBs and corrected mercury likely arose from the fact that PCB concentrations were correlated with both lipid and length, whereas mercury was more-strongly correlated with length and less-so with lipid. These contrasting examples demonstrate that correcting or adjusting data can change subsequent statistical results and possibly produce misleading conclusions. Statistical methods based on general linear models (such as ANOVA and ANCOVA) evaluate both simple and complex hypotheses that focus on linear relationships among variables. If hypotheses based on ratios are to be evaluated, randomization tests should be used instead of standard statistical tables. Randomization tests willverify the results of traditional analyses if the requisite parametric assumptions are met (i.e. normality, homogeneity of variance, and so on). Where these assumptions are violated, randomization tests will provide the correct probabilities to test the hypothesis of interest.

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WHAT LIMITS THE RATE OF METHYLMERCURY UPTAKE VIA THE GILLS OF FISH? G. Mierle, Ontario Ministry of the Environment, Dorset Research Centre, Dorset, Ontario.

The contamination of fish by mercury is a pervasive problem in inland waters (OMOE 1991), a phenomenon probably driven by widescale atmospheric deposition (Fitzgerald et al. 1991, Mierle 1990). A portion of this mercury is thought to be anthropogenic in origin (Andren and Nriagu 1979) and may be amenable to reduction by emission controls. However, the significant natural background of mercury, the lack of understanding of the large variability in the mercury concentration of fish between lakes, and the sensitivity of mercury accumulation to lake management practices make the development of models of the accumulation process an important goal for the control and prediction of this contaminant in fish.

Several models have been developed that relate methylmercury accumulation to energy metabolism (Fagerstrom et al. 1974, Norstrom et al. 1976). These models are based on the notion that methylmercury accumulation via the food and water are tightly linked to consumption and oxygen uptake, respectively. From an empirical standpoint the existence of these links is well established (Norstrom et al. 1976), but there are questions about the mathematical form of accumulation via gills.

Recent models of the accumulation of organic contaminants (Erickson and McKim 1990) via the gills endeavor to decompose this process into three components: movement of the contaminant to the gills by bulk water flow, diffusion of the contaminant across the gills, and removal of the contaminant from the gills by blood flow. The results of Erickson and McKim (1990) suggest that for some substances (e.g. organics with low binding affinity to blood proteins) all three steps may influence the uptake rate. For substances with high binding affinity removal by blood flow does not appear to be rate limiting. Because methylmercury has a very high binding affinity to blood proteins (Hughes 1957) it is very unlikely that blood flow is a limiting factor in methylmercury uptake.

Based on estimated values of a number of parameters Erickson and McKim concluded that when blood flow was not limiting, water flow was much more important than diffusion in limiting substance uptake. However, some of their parameters were based on assumptions that are difficult to validate independently. For example, estimates of diffusion area and distance were based on considerations of gill lamellae morphology. The effective values of these parameters, however, are very dependent on the patterns of water flow through or around the lamellae, the details of which are not observable. Hence, the relative importance of diffusion could easily be over or underestimated by a parameterization procedure based on morphology alone.

If we restrict our analysis to systems not limited by blood flow (e.g. where the internal concentration is maintained at very low levels by binding reactions) the analysis of uptake data can be considerably simplified if the ratio of uptake of two substances is considered. In the case of a diffusion limited system the terms for diffusion area and distance cancel out, and it can be shown that the relative of uptake efficiency is equal to the ratio of the diffusivities of the two substances of interest. For a water flow limited system the ratio of efficiencies should equal 1, and for mixed systems, i.e. flow and diffusion limited, the ratio should be intermediate.

Rogers and Beamish (1981) studied methylmercury uptake relative to oxygen uptake, and reported a relative efficiency of 0.25 for methylmercury uptake to oxygen uptake. This value is in precise agreement with the ratio of the diffusivities of methylmercury and oxygen and suggests that the uptake of methylmercury is diffusion, not flow limited. Rogers and Beamish also observed that the uptake of methylmercury relative to the concentration of methylmercury and oxygen could be well summarized by a power function which when linearized by log transformation had coefficients of 1.44 and 1.1 for the intercept and slope, respectively. (See their Fig. 5.) Assuming that the system is diffusion limited one can derive theoretical values for these coefficients. The value for the intercept depends on the oxygen gradient across the gills which was not determined, but with plausible estimates for their system the theoretical intercept would fall between 1.4 and 1.6. The slope should equal 1.0. Given reasonable allowance for error in their estimates these values are in quite good agreement.

The agreement between theory and observation in this analysis suggests that methylmercury uptake is limited primarily by diffusion. This conclusion differs from expectations based on Erickson and McKim's analysis. A possible explanation for this difference is an overestimate of the diffusion area of the gill and/or underestimate of distance in their study. Because of the much simpler nature of the analysis performed in the present study my conclusions may be more reliable.

If the present analysis is correct the results of Rogers and Beamish have a simple physical interpretation, and the application of their empirical equation for methylmercury uptake (or the theoretical equivalent) to fish in general is well justified. With the recent advent of reliable methylmercury concentration data in natural waters, the application of this model should resolve the question of the relative importance of water verse food uptake of methylmercury.

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BIOACCUMULATION DE MÉTAUX LOURDS CHEZ UN AMPHIPODE DU FLEUVE SAINT-LAURENT EN RELATION AVEC LA CONTAMINATION DE SÉDIMENTS.

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RESUMÉ

Notre étude a pour but d'évaluer le potentiel des amphipodes, organismes associés aux herbiers littoraux, en tant que bioindicateurs de la pollution métallique en milieu aquatique.

Des échantillons de sédiments de surface et des spécimens de l'amphipode *Gammarus fasciatus*, ont été prélevés en octobre 1990, à dix stations situées dans le lac St-Pierre, un élargissement du fleuve St-Laurent. Ces stations représentaient un gradient de pollution métallique et de matière organique dans les sédiments. Les amphipodes ont été digérés et le dosage du Cu, du Pb, du Zn, du Cd, du Ni, du Fe et du Mn a été réalisé par spectrométrie d'absorption atomique à la flamme et au four au graphite. Chaque échantillon de sédiments a été soumis à une procédure d'extractions séquentielles conçue pour déterminer la répartition de métaux traces entre diverses phases géochimiques.

L'analyse de régression indique que les teneurs en Ni, Pb et Fe dans les amphipodes sont mieux corrélés avec les fractions de métaux extraites relativement facilement des sédiments, ces fractions étant corrigées pour tenir compte de l'effet compétitif ou protecteur des oxyhydroxydes de Fe et de Mn amorphes, de l'importance relative du carbone organique et de la granulométrie des sédiments. Le Cu et le Zn restent à des niveaux relativement constants, suggérant une forme de régulation de leurs concentrations par les amphipodes.

INTRODUCTION

Les contaminants d'origine anthropique tendent à s'accumuler dans les sédiments à des concentrations 1000 à 5000 fois supérieures à celles de la colonne d'eau et présentent un risque écotoxicologique pour les organismes aquatiques et leurs prédateurs. La prédiction de la biodisponibilité des contaminants présents dans les sédiments aquatiques est nécessaire pour évaluer les dangers environnementaux que représentent les apports de contaminants en provenance des sources ponctuelles et diffuses (retombées atmosphériques) dans les écosystèmes aquatiques. Les modèles prédictifs de la bioaccumulation des polluants peuvent ensuite servir d'outils de gestion environnementale des niveaux de contamination dans les compartiments biologiques. Toutefois les prédictions de la biodisponibilité et de la bioaccumulation des contaminants dans les chaînes aquatiques sont liées à des processus complexes. En ce qui concerne les métaux lourds, plusieurs facteurs influencent leur incorporation et leur bioaccumulation par les organismes benthiques, soit les modes de nutrition des organismes, la répartition des métaux dans les phases géochimiques des sédiments (Campbell et Tessier, 1989) et les facteurs environnementaux (matières organiques, acidité, dureté, turbidité) (Jackson, 1986, 1988; Campbell et Stokes, 1985; Stephenson et Mackie, 1988).

Notre recherche a pour objectif d'évaluer le potentiel d'organismes associés aux sédiments de fond et au complexe macrophytes-épiphytes pour le développement d'indicateurs de qualité du milieu et de modèles de prédiction de la bioaccumulation des métaux dans les réseaux trophiques aquatiques. Dans ce but, nous voulons mettre en relation les quantités de Pb, Ni, Cu, Zn et Cd présentes chez *Gammarus fasciatus* (Crustacea; Amphipoda) avec la concentration de ces métaux lourds présents sous forme biodisponible au sein des sédiments, du seston et du périphyton de certains herbiers du lac St-Pierre, un élargissement du fleuve Saint-Laurent en aval de Sorel (Québec). Les amphipodes sont des organismes particulièrement intéressants à cause de leur importance en tant que ressources alimentaires de premier choix pour nombre d'espèces de poissons littoraux du fleuve Saint-Laurent (Fortin, 1970; Boisclair et Leggett, 1989).

Dans le cadre plus spécifique de cette présentation, nous nous bornerons à étudier la relation entre l'accumulation de métaux dans les amphipodes et la contamination des sédiments adjacents.

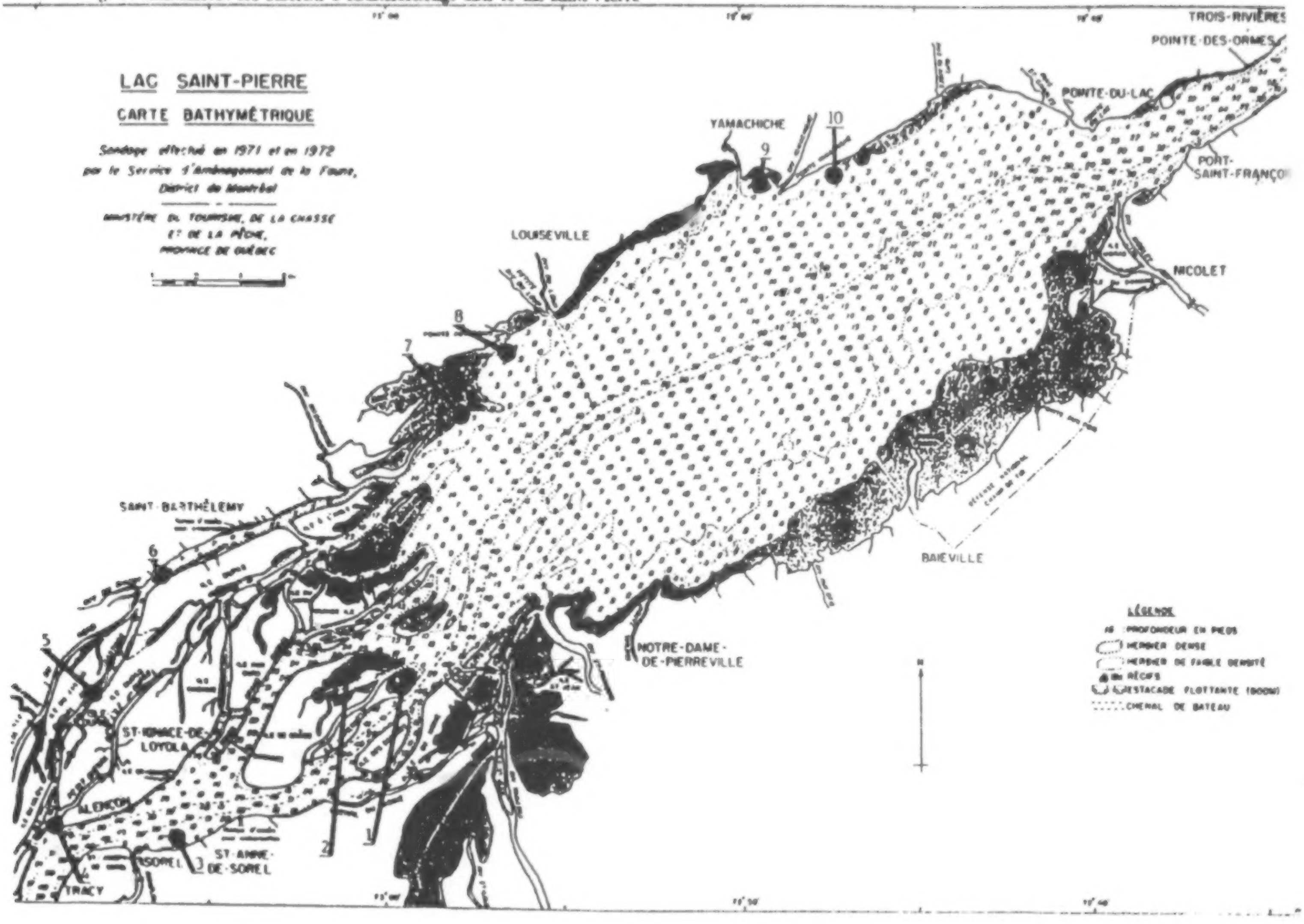
MATÉRIEL ET MÉTHODES

L'échantillonnage a porté sur une série de dix stations situées le long d'un gradient dans la concentration des métaux, la concentration en matière organique et la concentration en fer dans les sédiments. Pour tenir compte des variations naturelles des facteurs environnementaux (conductivité, pH, matière organique dissoute) dans ces milieux, nous avons choisi des stations dans les deux grandes masses d'eau en provenance du Saint-Laurent (eaux vertes) et des affluents du secteur nord du lac St-Pierre (eaux brunes de la rivière des Outaouais) (Figure 1). Lors des prélèvements et des analyses des échantillons, la verrerie entrant en contact avec les échantillons à doser était lavée à l'acide nitrique (15%). Lors des analyses, de l'acide ultrapure (qualité Aristar) et de l'eau déionisée (système Millipore) étaient utilisés.

Prélèvement et préparation des sédiments

A chaque station, nous avons prélevé des échantillons de sédiments, à l'aide d'un carottier en polypropylène. La partie superficielle oxydée (0.5 à 1 cm) était retirée à l'aide d'une tranche en Teflon, placée dans une bouteille à centrifugation en polypropylène, puis congélée à -10°C. Lors de l'analyse des métaux, la méthode de Tessier *et al.* (1979) a été utilisée. Après décongélation, les cailloux, les coquillages et les racines étaient écartés. Le reste des échantillons était alors centrifugé à 8000 tpm pendant 20 minutes et le surnageant était jeté. L'équivalent d'environ 1 g en poids sec de sédiment humide était introduit dans une éprouvette en Teflon. Par la suite, une extaction séquentielle conçue pour différencier différentes fractions géochimiques était effectuée dans l'ordre suivant: (a) M(F1) *métaux échangeables* : le sédiment était extrait pendant 30 min. dans du $MgCl_2$ 0.5 M à pH 7.0; (b) M(F2) *métaux liés aux carbonates ou spécifiquement adsorbés* : du NaOAc 1.0 M à pH 5.0 était ajouté au résidu de (a); (c) M(F3) *métaux liés aux oxydes de fer et de manganèse* : après un ajout au résidu de (b) de $NH_2OH.HCl$ 0.04 M dans HOAc 25 % (v/v), la suspension de sédiments restante était chauffée pendant 6 heures à 96°C; (d) M(F4) *métaux liés à la matière organique et aux sulfures* : le résidu de (c) était extrait à 85°C pendant 5 h après ajout de H_2O_2 30% à pH 2.0 (HNO_3), puis à la température de la pièce à l'aide de NH_4OAc 3.2 M dans HNO_3 20% (v/v). A la fin de chaque étape de la procédure, les surnageants étaient acidifiés si le milieu n'était pas déjà acide, puis dilués avec de l'eau Millipore.

Fig. 1. Localisation des stations d'échantillonnage dans le lac Saint-Pierre


LAC SAINT-PIERRE
CARTE BATHYMÉTRIQUE
Sondage effectué en 1971 et en 1972
par le Service d'Aménagement de la Faune,
District de Montréal
MINISTÈRE DU TOURISME, DE LA CHASSE
ET DE LA PÊCHE,
PROVINCE DE QUÉBEC
TROIS-RIVIÈRES
POINTE-DES-ORMES
POINTE-DU-LAC
PORT-SAINT-FRANÇOIS
YAMACHICHE
LOUISEVILLE
NICOLET
BAIEVILLE
SAINT-BARTHÉLEMY
NOTRE-DAME-DE-PIERREVILLE
ST-IGNACE-DE-LOYOLA
ALENÇON
SOREL
ST-ANNE-DE-SOREL
TRACY
1
2
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9
10
LÉGENDE
16 PROFONDEUR EN PIEDS
HERBIER DENSE
HERBIER DE FAIBLE DENSITÉ
RÉCIFS
ESTACADE FLOTTANTE (BOOM)
CHENAL DE BATEAU

Prélèvement et préparation des amphipodes

Les amphipodes étaient récoltés dans les macrophytes et les épiphytes de chaque station à l'aide d'un filet troubleau. Les organismes étaient par la suite divisés en trois classes de taille (3-7 mm, 7-9 mm et 9-12 mm). L'eau entourant les organismes était épongée à l'aide d'un papier buvard. Les individus d'une même classe de taille étaient placés dans de petites bouteilles en polypropylène, puis congelés jusqu'à l'analyse. Au laboratoire, les échantillons étaient séchés pendant 18 h à 70°C, puis pesés. Par la suite, ils étaient placés dans des bombes en Teflon. De l'acide nitrique Aristar était alors ajouté, et les bombes étaient placées dans un four à micro-ondes pendant 1.5 min à 360 W. Le digestat était par la suite dilué dans de l'eau Millipore pour l'obtention d'une concentration finale de HNO_3 12%.

Analyses des échantillons

Les analyses de métaux lourds ont été effectuées à l'aide d'un spectromètre d'absorption atomique à la flamme ou au four au graphite, dépendamment des concentrations présentes. Des échantillons certifiés (sédiments: MESS-1; hépatopancréas de homards: TORT (CNRC)) étaient utilisés pour valider la procédure analytique.

Physico-chimie

A chaque station, l'eau était analysée pour déterminer le pH, la turbidité, la couleur, la conductivité, le calcium dissous, l'oxygène dissous, la température et le carbone organique dissous. Les sédiments étaient analysés pour le pH, la teneur en eau, la concentration en soufre et en carbone organique. En plus, une étude granulométrique des sédiments a été effectuée.

RÉSULTATS ET DISCUSSION

Physico-chimie de l'eau

Tableau 1. Caractéristiques physico-chimiques de l'eau pour chaque station.

Station	Température (°C)	Oxygène (mg/L)	Conductivité (µmhos)	pH	Turbidité (UTJ)
1	12.5	9.0	411	7.34	5
2	13.0	9.4	260	7.30	5
3	12.0	8.4	327	7.20	21
4	12.0	8.9	164	6.82	15
5	11.5	9.6	127	7.07	10
6	11.0	8.2	199	7.02	39
7	10.0	10.1	173	7.10	19
8	11.0	10.0	141	7.11	32
9	6.5	10.4	121	7.03	70
10	5.0	11.3	101	6.84	40

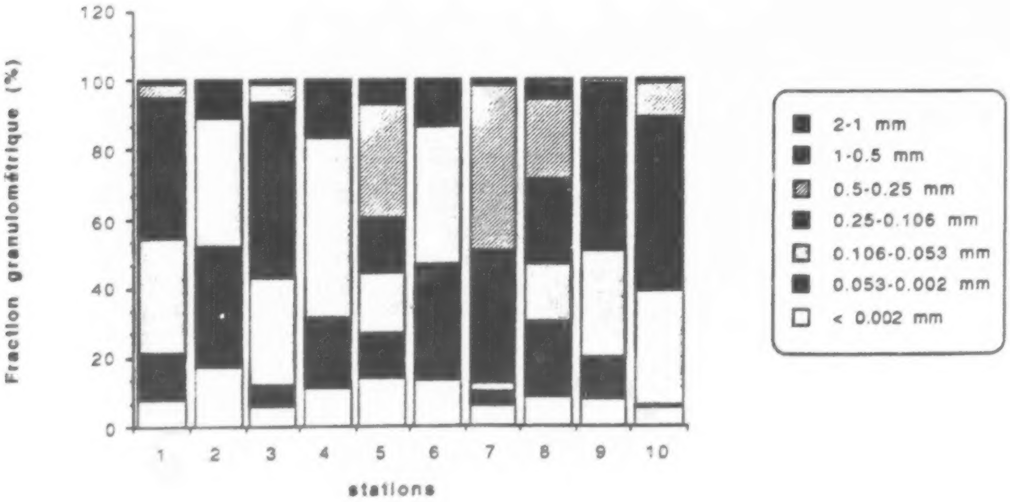
Le tableau 1 donne les principales caractéristiques physico-chimiques de l'eau aux différentes stations. L'emplacement des stations est indiqué sur la figure 1. On remarque d'une part que les stations situées dans la région nord du lac St-Pierre (stations 4,5,6,7,8,9 et 10) ont des valeurs de conductivité et de pH inférieures à celles recontrées dans le secteur sud (stations 1,2 et 3). Ceci s'explique par le fait que les eaux de la région nord du lac proviennent en grande partie de la rivière des Outaouais, laquelle possède des eaux brunes légèrement plus acides et moins minéralisées, alors que les stations de la région sud sont en contact avec les eaux vertes du fleuve St-Laurent plus riches en sels minéraux.

On remarque également de très hauts niveaux de turbidité aux stations 6, 9 et 10, lesquelles sont à l'embouchure de rivières aux eaux brunes (rivières Yamachiche et Chicot).

Physico-chimie des sédiments

Une étude granulométrique a été conduite au mois d'août 1990 aux mêmes stations que celles échantillonnées en octobre. Les résultats de cette étude sont résumés par la figure 2. D'une part, on remarque que les sédiments des stations échantillonnées ne comportent pas de gravier (portion supérieure à 2 mm) et peu de sable très grossier (de 2 à 1 mm) et grossier (de 1 à 0.5 mm) alors que les autres fractions granulométrique sont variablement représentées. D'autre part, les fractions argileuse (moins de 0.002 mm) et limoneuse (de 0.053 à 0.002 mm) se retrouvent en faible quantité aux stations 3,7 et 10. Le phénomène est encore visible, quoique moins prononcé, lorsque l'on fait l'intégration des fractions argileuse, limoneuse et du sable très fin (fractions inférieures à 0.106 mm). Or, en général, les concentrations de métaux dans les sédiments augmentent avec la proportion de sédiments fins. Plusieurs auteurs ont donc suggéré des procédures de correction pour l'effet de la granulométrie sur la variation de la contamination en métaux lourds entre différents sites. Entre autres, Klamer *et al.* (1990) ont comparé

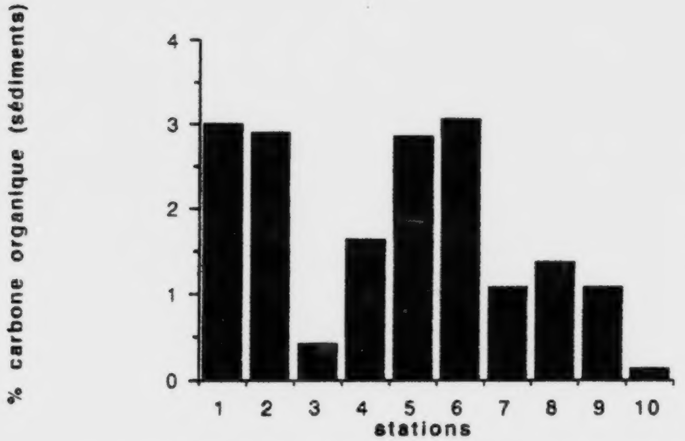
Fig. 2. Composition granulométrique des sédiments de surface pour chaque station.



différentes méthodes de correction en milieux marins et en sont venus à la conclusion que la meilleure procédure était la filtration des sédiments humides suivie de l'analyse des métaux dans la fraction inférieure à 0.063 mm. Il peut donc être pertinent de tenir compte de la granulométrie lors de l'élaboration de facteurs de correction lorsque l'on mettra en relation les concentrations de métaux dans les organismes avec celles des sédiments.

Par ailleurs, en tant qu'indice de la quantité de matière organique, le pourcentage de carbone organique dans les sédiments est une autre variable d'importance lors de la comparaison des concentrations de métaux lourds entre des sites ayant des sédiments différents. En effet, la matière organique, par des phénomènes d'adsorption, de complexation, de coagulation, de flocculation et autres, constitue un excellent piège des métaux lourds. La figure 3 montre la distribution du carbone organique dans les sédiments des différentes stations.

Fig. 3. Pourcentage de carbone organique dans les sédiments.



La distribution de carbone organique entre les stations est très hétérogène, des maxima évidents étant rencontrés aux stations 1,2,5 et 6 alors que les stations 3,7,9 et 10 montrent les pourcentages mimimums.

Contamination des sédiments

Les figures 4 à 9 montrent la répartition des métaux dans les différentes phases géochimiques des sédiments. Certains histogrammes sont incomplets, plusieurs valeurs se trouvant sous le seuil de détection analytique. De façon générale, on constate que l'objectif de retrouver un bon gradient entre les différentes stations est atteint. Notons que les concentrations en Cd étaient sous le seuil de détection pour les différentes fractions géochimiques.

En général, la répartition des métaux (c'est-à-dire, l'importance relative des fractions F1, F2, F3 et F4 pour chaque métal) semble conforme à celle que l'on retrouve habituellement dans la littérature pour les sédiments aérobies provenant de milieu d'eau douce. Ainsi, pour le Cu (Figure 7), la fraction F4 est prédominante, suivie par la fraction F3, ce qu'avait déjà constaté Chen *et al.* (1989), Tessier *et al.* (1980) et Viel *et al.* (1983). Une distribution semblable est présente dans le cas du Fe (Figure 4), quoique la prédominance de la fraction F3 soit plus prononcée, comme observé par Tessier *et al.* (1985). En ce qui concerne le Mn (Figure 5), la fraction F3

Fig. 4. Concentrations en Fe dans les différentes fractions géochimiques des sédiments.

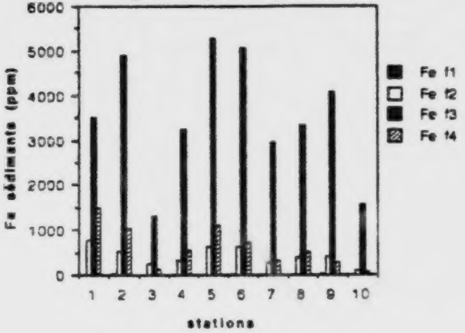


Fig. 5. Concentrations en Mn dans les différentes fractions géochimiques des sédiments.

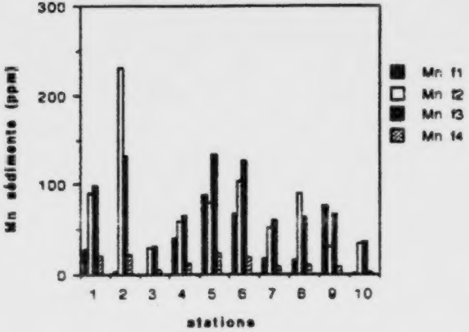


Fig. 6. Concentrations en Zn dans les différentes fractions géochimiques des sédiments.

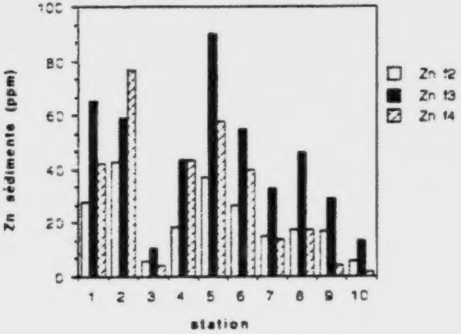


Fig. 7. Concentrations en Cu dans les différentes fractions géochimiques des sédiments.

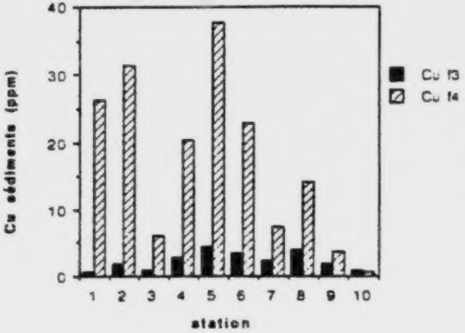


Fig. 8. Concentrations en Pb dans les différentes fractions géochimiques des sédiments.

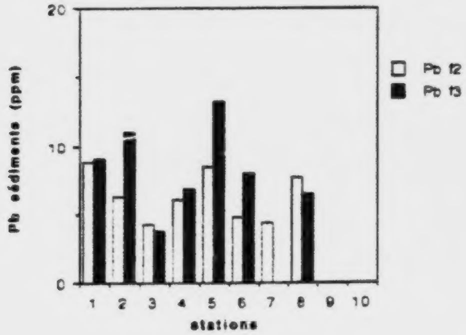
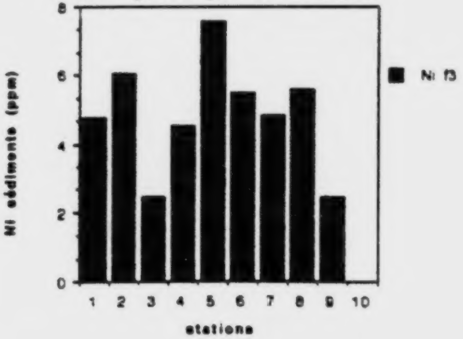


Fig. 9 Concentrations en Ni dans les différentes fractions géochimiques des sédiments.



domine, suvie de la F2 et de la F1. C'est pour ce métal que la fraction F1 semble la mieux représentée. Pour ce qui est du Zn (Figure 6), la fraction F3 domine encore, suivie de la fraction F2 ou F4, selon les stations. La prédominance de la fraction F3 n'est pas généralisée dans la littérature, quoiqu'elle soit souvent rencontrée (Tessier *et al.*, 1980; Viel *et al.*, 1983). Dans le cas du Pb (Figure 8), par contre, la forte contribution de la fraction extraite à l'acide acétique (pH 5.0) (F2), en association avec la F3, est plus rarement établie. Enfin, le Ni n'est dosable qu'au niveau de la fraction F3 (Figure 9). Notons également que la fraction des métaux directement échangeables (F1) est faiblement représentée, quelque soit le métal.

Au niveau des différences de contamination entre les stations, on remarque d'une part que les stations les moins contaminées sont les stations 3, 7,9 et 10 alors que les plus contaminées sont les stations 1,2,5 et 6. Les stations 4 et 8 sont plus ou moins contaminées, selon le métal. Il semble donc que les stations possédant les sédiments les plus grossiers et les quantités de carbone organique les plus faibles sont également celles où la contamination est minimale. On pourrait penser que ceci n'est qu'une coïncidence, mais le fait que la station 3, située immédiatement en aval des sources les plus importantes de contamination métallique du lac, fasse partie du groupe des stations les moins polluées au niveau des sédiments semble confirmer la validité de ces relations. De plus, notons que les stations 3 et 10 sont celles possédant les herbiers les moins denses. Il semble donc que le régime hydraulique de ces stations (présence de courants) ne soit propice, ni pour la sédimentation, ni pour la colonisation des macrophytes, ni pour la déposition des métaux lourds ce qui explique les caractéristiques physico-chimiques et toxicologiques des sédiments de ces stations.

Bioaccumulation des métaux dans les amphipodes

Les figures 10 à 16 indiquent les concentrations des différents métaux retrouvées dans les amphipodes, pour chaque classe de taille et pour chaque station. De façon générale, les concentrations obtenues sont du même ordre de grandeur que celles rencontrées dans les sédiments.

Les concentrations en Zn (Figure 12) semblent relativement constantes peu importe les stations, laissant supposer une certaine forme de régulation pour ce métal qui est un constituant essentiel pour le métabolisme animal. D'ailleurs, d'autres études ont déjà fait état d'une certaine régulation chez les amphipodes (Amiart *et al.*, 1987; Rainbow et White, 1989; Rainbow *et al.*, 1990). Pour ce qui est du Cu, un autre constituant essentiel présentant des niveaux de bioaccumulation assez constants, l'hypothèse de la régulation est problématique, puisque les légères variations rencontrées en octobre avaient déjà été observées au mois d'août précédent (données non publiées). Il est donc probable que les concentrations de Cu dans les organismes soit dépendantes de certaines caractéristiques du milieu. Malgré tout, le fait que les variations observées soient relativement faibles laisse croire qu'une régulation plus ou moins efficace du Cu agit. Notons, par ailleurs, que l'hypothèse de l'accumulation du Cu semble être appuyée par plusieurs études conduites sur d'autres espèces marines d'amphipodes. Par exemple, Rainbow et White (1989) ont trouvé que pour les amphipodes *Echinogammarus pirloti et E. modestus*, il y avait accumulation de Cu à toutes les concentrations de Cu dissous avec aucune indication de régulation. Des études plus approfondies, en laboratoire, devraient être conduites pour déterminer l'importance de la régulation du Cu chez *Gammarus fasciatus*. Pour le Fe, le Mn, le Pb, le Ni et le Cd, les variations rencontrées sont prononcées et toute régulation peut être écartée (Figures 10,14, 15 et 16).

Par ailleurs, même si les variations entre les différentes classes de taille ne sont pas très marquées, l'on constate que, dans le cas du Fe, Mn, Pb et Cd, la classe de

Fig. 10. Concentrations en Fe dans les amphipodes pour chaque classe de taille

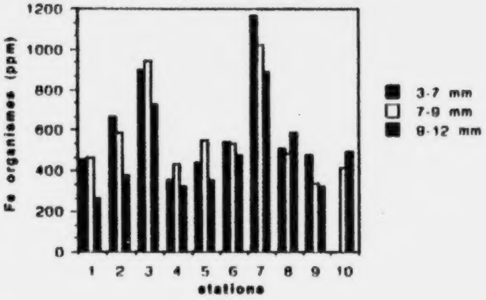


Fig. 11. Concentrations en Mn dans les amphipodes pour chaque classe de taille

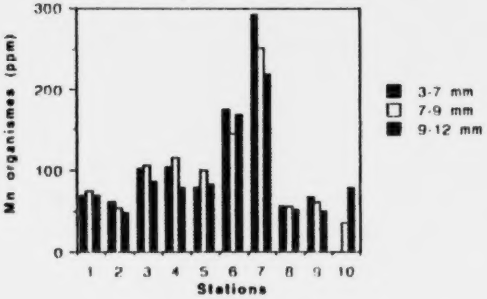


Fig. 12. Concentrations en Zn dans les amphipodes pour chaque classe de taille

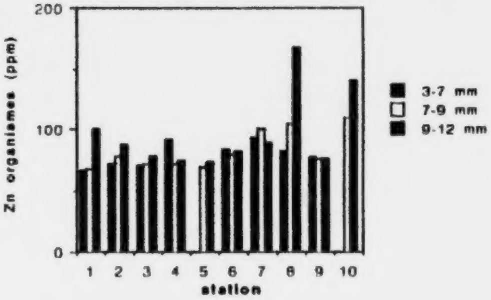


Fig. 13. Concentrations en Cu dans les amphipodes pour chaque classe de taille

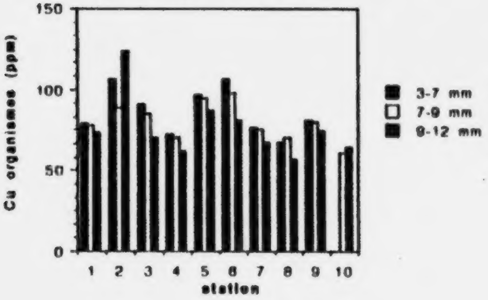


Fig. 14. Concentrations en Pb dans les amphipodes pour chaque classe de taille

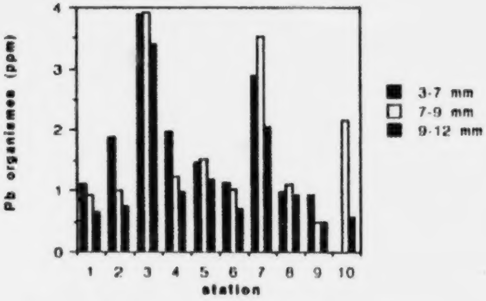


Fig. 15. Concentrations en Ni dans les amphipodes pour chaque classe de taille

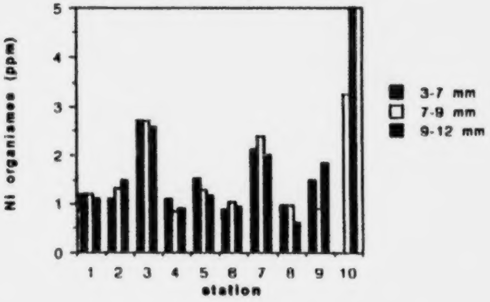
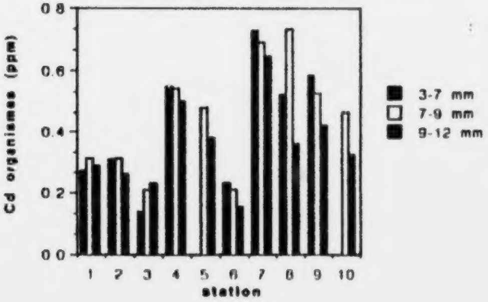


Fig. 16. Concentrations en Cd dans les amphipodes pour chaque classe de taille



taille la plus grande est la moins contaminée, dans 70 à 100% des stations, dépendemment du métal. Ceci est consistant avec les relations habituellement trouvées entre la taille et l'accumulation de métaux chez les amphipodes (Rainbow et Moore, 1986). Par contre dans le cas du Zn, seulement deux stations montrent cette situation, renforçant l'hypothèse d'un certain contrôle homéostatique du Zn par les amphipodes.

Finalement, l'examen des concentrations de métaux chez les amphipodes de différentes stations montre que les concentrations maximales dans les organismes se rencontrent souvent aux stations qui affichaient des concentrations minimales dans les sédiments. C'est en particulier le cas pour le Fe, le Pb et le Ni où les stations 3,7 et 10 présentent les plus fortes contaminations animales. Le Mn et le Cd ont également un pic à la station 7 dont les sédiments sont peu contaminés. Les concentrations rencontrées aux stations 1,2,4,5,6,8 et 9 sont souvent semblables dans le cas du Fe, Zn, Pb et Ni.

Relation entre la contamination des sédiments et celle des organismes

Dans un premier temps, l'ensemble des relations pouvant exister entre les concentrations des différents métaux dans les organismes et celles dans les différentes fractions géochimiques des sédiments ont été vérifiées par des tests de corrélation (r de Pearson) (Tableau 2).

Tableau 2. Coefficients de corrélation (r de Péarson) entre les concentrations de métaux dans les sédiments et celles dans les organismes.

Métal	classe (mm)	MF1	MF2	MF3	MF4	MF3/a	MF3/a*b*c
Mn	3-7	-0.093	-0.278	-0.225	-0.348	-0.189	0.701*
	7-9	0.082	-0.316	-0.188	-0.269	-0.312	0.120
	9-12	0.087	-0.195	0.007	-0.117	-0.139	0.123
Fe	3-7	0.181	-0.520	-0.456	-0.496	0.032	0.895***
	7-9	0.113	-0.420	-0.315	-0.413	0.063	0.873***
	9-12	0.037	-0.605*	-0.463	-0.649*	0.195	0.886***
Cu	3-7	--	--	0.033	0.456	-0.268	-0.271
	7-9	--	--	0.070	0.593*	-0.246	-0.554*
	9-12	--	--	0.125	0.553	-0.468	-0.370
Zn	3-7	--	-0.338	-0.233	-0.235	0.098	0.510
	7-9	--	-0.513	-0.464	-0.553*	-0.412	0.430
	9-12	--	-0.305	-0.319	-0.341	-0.280	-0.165
Ni	3-7	--	--	-0.474	--	0.365	0.853***
	7-9	--	--	-0.199	--	0.556*	0.883***
	9-12	--	--	-0.556	--	0.368	0.734*
Pb	3-7	--	-0.723*	-0.621	--	0.087	0.910***
	7-9	--	-0.728**	-0.765**	--	-0.366	0.940***
	9-12	--	-0.632	-0.641	--	0.161	0.983***

* $p < 0.05$: ** $p < 0.01$: *** $p < 0.001$

MFx: métaux liés à la fraction x

Facteurs de correction: a: concentrations en Fe dans la F3 (ou en Mn, lorsqu'utilisé pour le Fe)
b: % de sédiments inférieurs à 0.106 mm
c: % en carbone organique dans les sédiments

Un grand nombre de relations négatives ont alors été observées, lesquelles étaient rarement significatives. Dans les quelques cas où des relations positives furent décrites, les coefficients étaient bas. Seul dans le cas du Cu, une relation significative et positive a été obtenue entre les concentrations dans les organismes et celles dans la fraction F4. Par la suite, l'ensemble des concentrations de métaux dans chacune des phases géochimiques a été normalisé en fonction des concentrations d'oxyhydroxydes de Fe et de Mn amorphes, lesquels sont reconnus comme exerçant un rôle protecteur ou compétitif (Campbell *et al.*, 1988). Les corrélations furent alors plus souvent positives, mais rarement significatives (Tableau 2). C'est le Ni qui semble le plus avoir bénéficié de cette correction.

Finalement, après ajout au facteur de correction d'une variable représentant la granulométrie et d'une autre associée à la matière organique, de très fortes corrélations sont ressorties dans le cas du Fe, du Ni et du Pb (Fig. 17 A,B et C; Tableau 2). Les résultats ainsi obtenus comportent certains problèmes d'interprétation. D'une part, les diagrammes de dispersion (e.g. fig 17 A, B et C) montrent souvent des agglomérations de points de part et d'autre de la droite de régression. Pour valider ces relations, il serait donc fort utile d'obtenir des données provenant de plusieurs autres sites afin d'augmenter l'effectif de l'échantillon. D'autre part, le facteur de correction composite comprend des variables qui sont autocorrélées. Ainsi, les corrélations obtenues entre l'importance relative des différentes fractions granulométriques et le pourcentage de carbone organique sont significatives ($p<0.05$) pour la fraction la plus fine (Tableau 3). De même, les concentrations d'oxyhydroxydes de Fe et de Mn amorphes sont significativement corrélées avec les fractions granulométriques les plus fines et avec le pourcentage de carbone organique (Tableau 3).

Tableau 3. Coefficients de corrélation (r de Pearson) entre différentes caractéristiques physico-chimiques des sédiments.

	MnF3	% C org.	%0.053-0.002 mm	%<0.002 mm
FeF3	0.925***	0.790**	0.714*	0.844**
MnF3		0.861**	0.724*	0.884***
% Corganique			0.566	0.787**
% 0.053-0.002 mm				0.845**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

L'emploi simultané de ces trois variables au sein d'un même facteur de correction peut donc avoir tendance à grossir artificiellement le coefficient de corrélation. Par contre, il se peut que ces variables aient des effets additifs sur la rétention des métaux dans les sédiments et qu'il soit ainsi important de les traiter simultanément pour obtenir de bonnes relations. L'approche qu'il faudrait privilégier est une analyse factorielle permettant d'évaluer l'importance relative des différentes variables dans la "structure" physico-chimique des stations; le nombre restreint de stations limite l'emploi d'une telle méthode actuellement.

Par ailleurs, l'effet possible du pH sur la biodisponibilité des métaux n'a pu être correctement étudié, étant donné que les variations de pH entre les stations à cette période de l'année étaient faibles. Or, l'on sait que d'autres études ont déjà souligné l'importance de ce facteur sur la biodisponibilité des métaux (Campbell et Tesssier, 1989).

De façon générale, les concentrations de métaux dans les différentes

Fig. 17 A. Relations entre les concentrations en Fe dans les amphipodes et celles dans les sédiments, après correction.

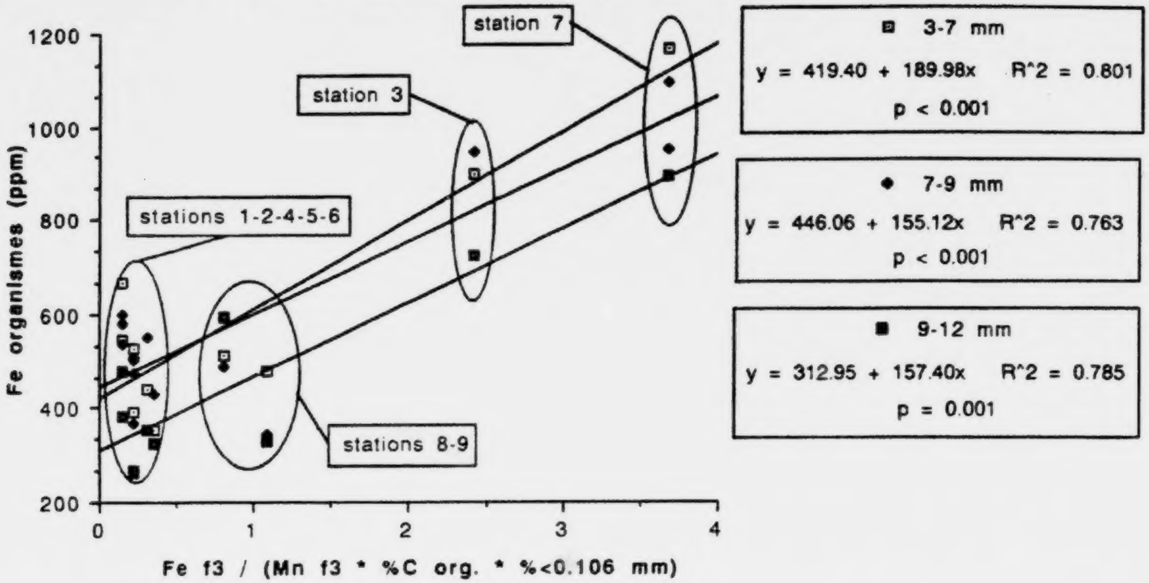


Fig. 17 B. Relations entre les concentrations en Ni dans les amphipodes et celles dans les sédiments, après correction.

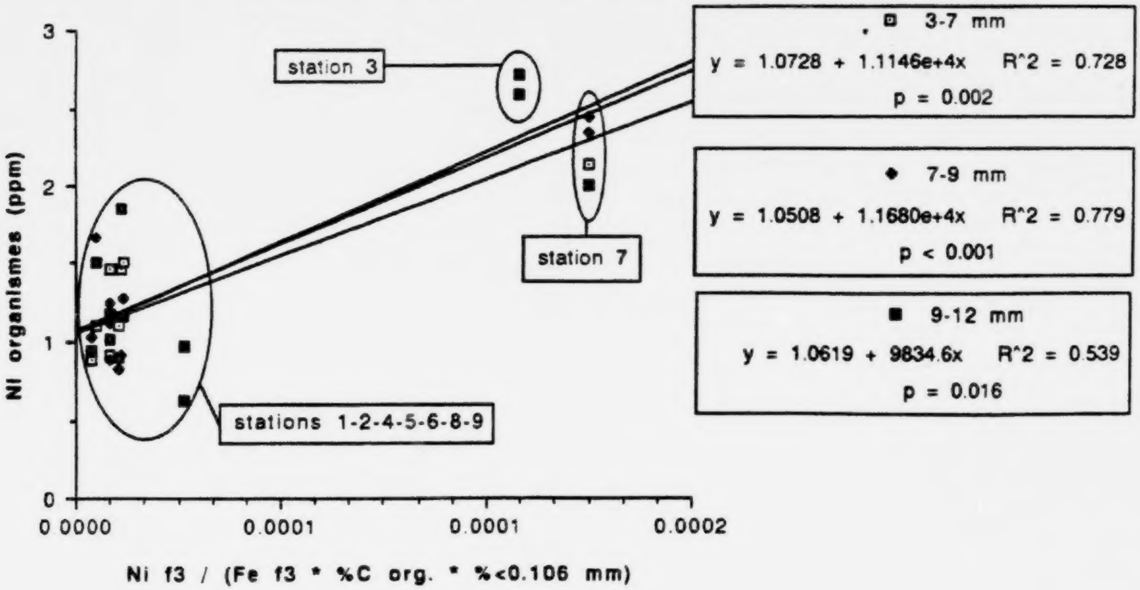
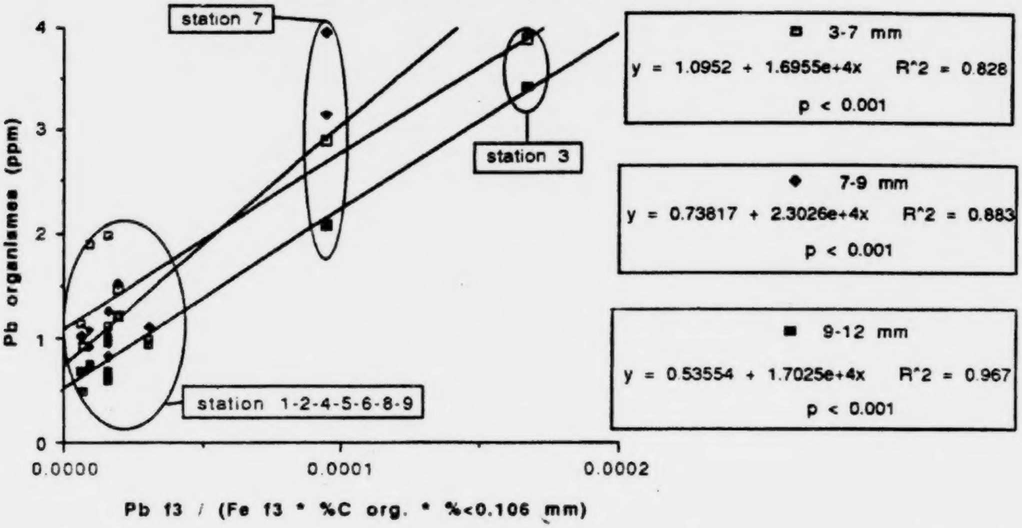


Fig. 17 C. Relations entre les concentrations en Pb dans les amphipodes et celles dans les sédiments, après correction



fractions géochimiques des sédiments ne semblent pas directement corrélées avec les concentrations dans les organismes, même après une correction pour l'effet compétitif des oxyhydroxydes de Fe et de Mn. Ceci semble indiquer que le processus de bioaccumlation des métaux lourds chez les amphipodes n'est pas principalement axé sur l'absorption de métaux relargués des sédiments ou l'ingestion de sédiments contaminés. D'ailleurs, une analyse des tractus intestinaux a souligné l'absence de sédiments dans les fécès de ces organismes.

Il est fort possible que ce soit par l'intermédiaire de plusieurs autres voies de bioaccumulation comprenant l'ingestion de métaux par la nourriture et l'absorption cutanée des métaux dissous dans l'eau que la contamination se fasse. Dans le but d'éclaircir l'importance relative de ces voies, des analyses ont été conduites aux mêmes stations afin de déterminer la contamination du périphyton, du seston et des macrophytes, ces éléments rentrant tous dans la diète de *Gammarus fasciatus*. Par la suite, un modèle de prédiction construit de façon similaire à celui proposé par van Hattum *et al.* (1991) sera ébauché. Ces auteurs ont élaboré un modèle tenant compte à la fois des composantes abiotiques et biotiques, obtenant des prédictions respectables dans le cas du Cd et du Cu chez *Gammarus pulex*.

En conclusion de cette étude, il semble donc que la bioaccumulation des métaux lourds chez l'amphipode *Gammarus fasciatus* ne soit pas hautement influencée par le relarguage des métaux dans les sédiments. L'emploi de cet amphipode en tant que bioindicateur de la qualité des sédiments semble donc restreint, à moins de tenir compte de la granulométrie, de la matière organique et des teneurs en oxyhydroxydes de Mn et de Fe dans les sédiments.

REMERCIEMENTS

Cette étude a été rendue possible grâce à l'assistance financière du Conseil de recherches en sciences naturelles et en génie du Canada et du Centre Saint-Laurent (subvention partenariat à B. Pinel-Alloul et P.G.C. Campbell) et grâce à une bourse de 2^e cycle du CRSNG à M. Amyot. Nos remerciements vont aussi à Jean-Pierre Amyot, Ginette Méthot, Christiane Flessas et Joël Désy pour leur aide technique.

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PROBLEMS IN THE USE OF BIOLOGICAL INDICATORS OF METAL CONTAMINATION.

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The rationale behind the use of organisms to assess levels of metal contamination is discussed. Because different species accumulate metals from a variety of sources, and because no single species is ideal for every element, there is clearly no universal indicator organism. To be truly meaningful, monitoring programmes should involve analysis of several ecological types, for example algae (dissolved metal), suspension feeder (suspended material), deposit feeder (benthic sediment) and carnivore (food chain magnification).

In UK estuaries biological availability of most metals differs by orders if magnitude between highly contaminated and pristine sites and, generally, the quantity and form of the metal (abiotic factors) dominate the degree of bioaccumulation. The ability to reflect such changes in bioavailability is clearly over-riding importance in selecting indicator organisms. However, biotic factors (age, size, metabolism and transformation of metals etc.) can also modify this selection and may be highly relevant in interpreting the biological significance of tissue burdens. Thus, the induction of metal detoxification systerms, for example, through ameliorating toxic effects, may lead to exceptionally high tissue concentrations.

VARIATION OF Cd BODY BURDEN CONCENTRATIONS AND BURDENS IN AQUATIC INVERTEBRATES.

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This study examines the relationship between body size and Cd concentration in several taxa of freshwater invertebrates. The slope of the body burden - body size relationship was >1 in some taxa, indicating that concentration increased with body size. In other taxa, the slope was <1, indicating a decrease in concentration with body size. The importance of various factors other than body size, such as life cycle length and methods used to determine metal concentrations in the samples, was also examined.

DEGRADATION DU CHLORURE DE TRIBUTYLÉTAIN PAR L'ALAGUE MARINE *Pavlova lutheri*; EFFETS SUR LA CROISSANCE DE L'ALGUE.

R. St-Louis, E. Pelletier, P. Marsot et R. Fournier, Centre Océanographique de Rimouski, Rimouski, Québec.

L'utilisation du tributylétain et ses dérivés, pour la protection des coques de bateaux et des installations immergées a mené à la pollution locale des environnements côtiers. L'écotoxicité de ces composés se remarque à des concentrations allant du µg/L au ng/L selon l'organiame marin étudié et le test de toxicité utilisé. Pour le phytoplancton marin ces valeurs sont souvent obtensues sur des cultures en enceintes closes; ainsi la croissance de *Pavlova l.* est stoppée après 2 jours pour une contamination du milieu en tributylétain de 5 µg/L. Nous avons réévalué l'impact de ce polluant sur *Pavlova lutheri* en maintenant une population d;algues en culture continue, i.e. avec unrenouvellement du milieu de culture. Nous remarquons alors que la population est beaucoup plus tolérante au polluant et semble même en tirer profit. Pour une concentration de 5 µg/L en chlorure de tributylétain la croissance de l'algue n'est pas affectée. Après 2 jours il y a une accumulation intracellulaire de l'organoétain où il y est dégradé en dibutylétain. De ce travail il semble ressortir que la concentration létale pour cette algue soit plus importante que celle déterminée lors d'études en enceinte close. Certains aspects physiologiques de la réponse de l'algue au polluant sont abordés.

SESSION 1C

WHOLE ECOSYSTEM ASPECTS OF CONTAMINANT TRANSFER/ASPECTS ÉCOSYSTÉMIQUES GLOBAUX DU TRANSFERT DES CONTAMINANTS

CHAIRPERSONS/PRÉSIDENTES
John Uthe and David Lean

RELATIVE IMPORTANCE OF MACROPHYTE SPECIES AS CADMIUM SINKS IN A SHIELD LAKE RECEIVING EXPERIMENTAL ADDITIONS OF CADMIUM.

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EXTENDED ABSTRACT

Cadmium is a heavy metal, distributed by long-range transport of air pollutants and by liquid effluent emissions, that is highly toxic to aquatic life. Toxicity of Cd is highest in waters of low hardness. The Canadian Water Quality Guideline (CWQG) for the protection of aquatic life in waters of less than 60 mg/L hardness is set at 200 ng/L. A whole lake experimental addition of Cd was initiated in 1987 in Precambrian Shield Lake 382 in the Experimental Lakes Area, northwestern Ontario, to study the geochemical and biological pathways and the adverse effects of low levels of Cd in a very soft water lake by observing the responses of the lake ecosystem to Cd concentrations up to and including the CWQG. The accumulation of Cd in and the effects of Cd on a number of compartments, including the macrophytes, are being studied.

Cadmium labelled with ^{109}Cd was added to Lake 382 to raise the epilimnetic [Cd] from 1.6 ng/L, prior to experimentation, to 100 ng/L in 1987 and 1988 and to 120 ng/L in 1989. Actual measured concentrations in the ice-free seasons averaged 83, 50 and 100 ng/L in 1987, 1988 and 1989, respectively.

In July 1989, standing biomass of 14 of the 18 species of macrophytes present was estimated, as well as the Cd sequestered by each species. Total wet standing macrophyte biomass for the lake was estimated as 27000 kg. This is 14.6 times the wet standing algal biomass in the euphotic zone (D. Findlay, Freshwater Institute, pers. comm). Eriocaulon septangulare, Isoetes echinospora, Lobelia Dortmanna and Sagittaria sp. contributed 49, 14, 18, and 15%, respectively, or a total of 96%, to the standing macrophyte biomass. Live biomass of the floating species, Nuphar variegatum and Potamogeton natans as only 1.5 % of total standing biomass and the emergents, such as Carex spp contributed 3.5%.

The species accumulating Cd (μg Cd/g tissue, calculated from ^{109}Cd accumulation) to the greatest extent were Utricularia > Nuphar variegatum > Myriophyllum verticillatum. Also, the roots of Carex lacustris showed high accumulation, although the shoots showed little or no accumulation. Among the macrophytes, E. septangulare was the largest sink for the added Cd, containing about 3.5 g of the 2000 g added to the lake. Lobelia Dortmanna and I. echinospora contained less than 0.5 g Cd each and Sagittaria sp., less than 0.2 g. The other species were minor sinks.

The sediment is the major sink for Cd in Lake 382 containing an estimated 90-95% of the Cd added by any time. The macrophyte community, although containing only about 4% of the added Cd, is a larger Cd sink than fish and macrobenthic communities.

A REGIONAL ASSESSMENT OF MERCURY CONTAMINATION IN YELLOW PERCH (Perca flavescens) IN ONTARIO: IMPLICATIONS FOR COMMON LOONS (Gavia immer).

I.D. Cuthbert, McGill University, Departemt of Biology, Montéal, Québec.

Abstract

Yellow perch were used to assess the potential for dietary exposure to mercury at levels associated with adverse biological effects in common loons. Data from government surveys were used to estimate standard length dorsal tissue mercury concentrations in 20 and 25 cm yellow perch in 57 Ontario lakes. Previous work in contaminated systems has shown that average mercury concentrations greater than 0.3-0.4 ppm in loon prey is associated with reduced egg laying and nest site and territorial fidelity. Although none of the lakes in this study are known to receive point source mercury contamination, the estimated mercury levels of 20 cm yellow perch in 14 percent of the lakes studied exceeded 0.30 ppm. This proportion increased to 42 percent of the lakes for 25 cm perch. These results suggest that mercury concentrations in prey at levels potentially harmful to common loon reproduction is widespread in Ontario. These findings are expected to extend to large areas of Eastern North America, with similar geologies and atmospheric acid deposition levels.

1. Introduction

In recent years, massive die-offs of common loons (*Gavia immer*) have been observed in their wintering grounds along the southeastern United States coast. Although these deaths have been attributed directly to parasites, it has been widely speculated that mercury toxicity had an important role in the loons' exposure to, and susceptibility to infestation [1-4]. Toxic effects of mercury on birds include the impairment of vision and motor control, both critical to the loons' skill in finding and capturing fish. It was suspected that mercury contamination may have reduced the loons' hunting ability, forcing them to switch from a fish to a crab diet, thereby encountering the parasites [2; 4]. Laboratory analyses of loon carcasses collected along the Gulf of Mexico coast during the 1983 die-off revealed that the majority of the birds had tissue mercury concentrations at or near the lethal level [1]. These findings founded the hypothesis that the entire North American common loon population is affected by mercury contamination [1]. However, there is little solid evidence to support this claim, and the hypothesis remains untested.

Loons and other fish-eating birds occupy the top niche in the aquatic food web, and as such, they are particularly vulnerable to exposure to bioaccumulative, lipophilic contaminants, including mercury. However, despite the extensive work that has been done to quantify the extent and the dynamics of mercury contamination in aquatic and terrestrial ecosystems, very little attention has been focused on the common loon. It is likely that the reason for this dearth of information is linked to the public's perceived value of this species, and its protection by law. As a result, researchers may be either reluctant or unable to obtain adequate samples with which to assess the extent and effects of mercury contamination on loon populations. However, numerous studies have been done on mercury levels in fish, and these data may provide the best means for predicting loon mercury levels, without requiring the sacrifice or disturbance of any birds. Loons are territorial birds, and are known to return to the same lakes every summer, for as long as 10 years [4]. This territorial behavior provides a direct link between specific lake water quality conditions, (including contaminant levels in prey) and individual birds, throughout their lifetimes. Previous research has clearly demonstrated that mercury levels in loons are positively correlated with those of their prey in specific territories [2].

Barr (1986) found that mercury concentrations of 0.30-0.40 ppm and greater in prey are associated with failure in loon egg laying, and reduced nest site and territorial fidelity [2]. In the present study, the levels of mercury in yellow perch (*Perca flavescens*) were used to estimate the regional extent of mercury contamination in prey at levels potentially harmful to common loon reproduction (i.e., > 0.30 ppm). This study is limited to the province of Ontario, as comparable data on the concentrations of mercury in yellow perch are not currently available elsewhere.

Methods

The diet of common loons in Ontario lakes is varied, but their most common prey is yellow perch [2,4]. Yellow perch are often the most abundant suitably-sized prey occuring in these lakes, and they are well distributed throughout all but the most northwestern region of Ontario [5]. In addition to their abundance, yellow perch are also relatively easy prey for diving birds to catch. Their tendency remain in the epilimnion, where light conditions are optimal for diving loons, coupled with their erratic swimming behavior during pursuit, make them considerably easier to capture than other, similarly-sized pelagic fishes [6].

Loons are known to feed on fish of varying sizes, from 10-50 cm in length. Feeding experiments have shown that common loons prefer small yellow perch (10-25 g, or approximately 10-14 cm) to larger prey. However, they will also consume larger, more elusive prey (70-110 g, or approximately 18-21 cm) even when the smaller individuals are present [6]. Loons have been observed consuming considerably larger prey. For example, the stomachs of 3 accidentally drowned loons feeding on stocked trout (Salmonidae) in New York lakes contained 18 trout, with an estimated average total length of 25 cm. This included one trout approximately 50 cm long, weighing 500 g [4].

For the purposes of this study, standard lengths of 20 cm and 25 cm have been selected to quantify the concentrations of mercury in yellow perch tissues. It assumed that in most lakes, perch of this size have made the transition from planktivorous to primarily piscivorous feeders, although there may be considerable variation in their diets between lakes [5]. Because of the bioaccumulative nature of mercury, the change in the trophic position of 20 cm and larger yellow perch should have a direct effect on tissue mercury concentrations. Previous work has shown that there is an abrupt increase in the rate of mercury accumulation in fish tissues associated with the switch from an invertebrate to a vertebrate diet, which also corresponds to an increase in growth rate [7].

Data on the mercury concentrations in dorsal muscle tissue of 839 yellow perch from 57 Ontario lakes were obtained from government surveys [8,9]. Total mercury concentrations in yellow perch (expressed on a wet weight basis) have been standardized to 20 cm and 25 cm total length. Information for 13 of the lakes was limited to summary data, which included length-[Hg] equations for each lake [8]. The provided equations were used to calculate mercury concentrations in muscle tissues of standard length perch on a lake by lake basis. Standardized length [Hg] for perch from the remaining 44 lakes [9] were calculated using separate total length-[Hg] regression equations for each lake. These equations were developed from raw data on mercury levels in perch. A typical example of the relationship between fish length and [Hg] is presented in Figure 1. Linear regressions were calculated using microcomputer SYSTAT version 5.0.

The catchment geologies of the 57 lakes were ranked according to their ability to buffer acidity. Catchments scored 0 (low buffering capacity) to 6 (high buffering capacity) using information from government surveys [10]. This approach was required in the absence of sufficient lake pH data.

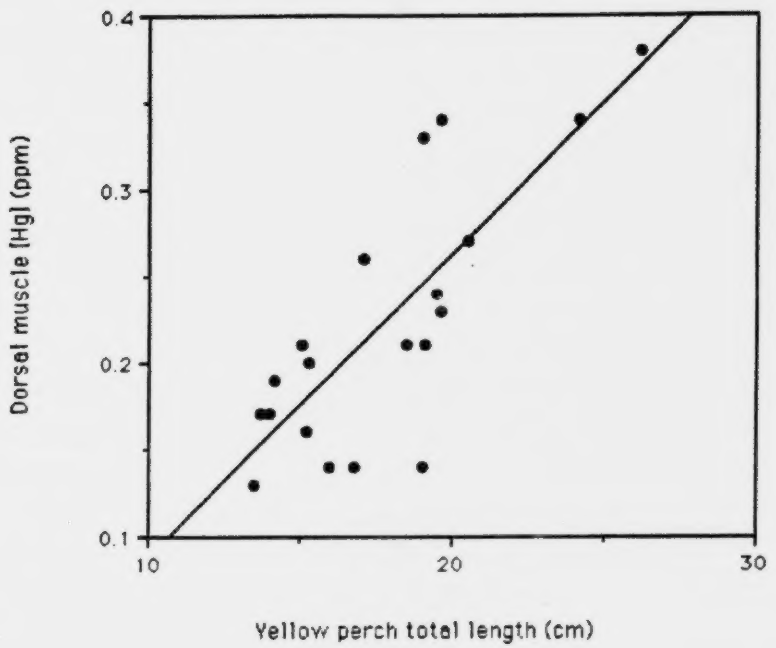

Figure 1. The relationship between yellow perch total length and dorsal muscle tissue [Hg]. Data from Round Lake yielded the linear regression [Hg] ($\mu g\ g^{-1}$) = 0.0174 total length (cm) - 0.087 ($R^2 = 0.62$, $N = 20$, $SE_{est} = 0.048$).

Results

The lakes included in this study, their geographical coordinates, and the mercury concentrations of standard length 20 cm and 25 cm yellow perch in the 57 lakes are presented in Table 1. Summary statistics are presented in Table 2.

The average size of the 602 perch sampled in 44 lakes [9] was 20.5 cm (minimum = 12.1 cm, maximum = 35.4 cm), with an average weight of 130.2 g (minimum = 14.0 g, maximum = 550.0 g). Dorsal muscle mercury concentrations in the yellow perch ranged from 0.01 - 1.50 ppm, with a mean concentration of 0.234 ppm. The number of yellow perch included in the lake samples ranged from 3 - 25, with an average of 15 fish. The strength of the length versus [Hg] linear regression relationships varied considerably between lakes.

Table 1. Locations of the 57 Ontario lakes included in the study. Also given are the number of yellow perch sampled in each lake (N), the strength of the length-[Hg] relationship (R^2), and the standardized total mercury concentrations in dorsal muscle tissues of 20 cm and 25 cm yellow perch. Mercury concentrations are reported as ppm wet weight. Length-[Hg] equations were provided (*) from a previous study for 13 lakes [8].

Lake	Lat.	Long.	N	R^2	[Hg] 20 cm	[Hg] 25 cm
Arran	4429	8115	20	0.60	0.169	0.246
Balsam	4435	7850	20	0.35	0.130	0.188
Beaton	4853	8448	3	0.97	0.328	0.450
Belmont	4431	7749	20	0.48	0.505	0.607
Bennett	4455	7628	8	0.10	0.039	0.143
Cameron	4433	7846	13	0.25	0.212	0.261
Canal	4434	7903	20	0.52*	0.126	0.159
Chemung	4424	7824	16	0.85*	0.151	0.242
Constan	4524	7659	13	0.25	0.226	0.325
Couchiching	4440	7922	25	0.61	0.147	0.246
Dore	4537	7707	8	0.61	0.204	0.330
Dumbell	4449	7934	15	0.48	0.293	0.407
Eugenia	4419	8030	16	0.38	0.211	0.257
Farren	4446	7630	14	0.32	0.171	0.217
Hidden	4844	8050	10	0.62	0.060	0.129
Isaac	4447	8114	30	0.61	0.131	0.189
Jackfish	4850	8657	7	0.89	0.224	0.571
Jordan	4504	7804	23	0.41	0.436	0.698
Kamaniskeg	4525	7741	5	0.35	0.205	0.257
Katchewanooka	4427	7816	20	0.33	0.139	0.194
L. Buckhorn	4432	7818	15	0.65	0.204	0.299
Lac des Milles	4850	9030	8	0.77	0.041	0.167
L. of the Woods	4853	9440	21	0.70*	0.074	0.205
Lillabelle	4906	8102	12	0.29	0.099	0.109
Little Cedar	4841	8550	6	0.72	0.101	0.169
Long	4448	7615	14	0.60	0.137	0.204
Lutterworth	4452	7850	12	0.85	0.432	0.765
Margaret	4916	8108	4	0.76	0.258	0.524
Mawn	4908	8914	20	0.84*	0.226	0.389
Mississaganon	4452	7705	15	0.44	0.151	0.195
Muskeg	4900	9002	5	0.93	0.237	0.409
Nippissing	4610	7950	15	0.62*	0.222	0.345
Opinicon	4434	7619	7	0.93	0.270	0.430
Pigeon	4427	7830	20	0.61*	0.075	0.115
Poplar	4640	7937	15	0.62*	0.207	0.351
Rice	4412	7810	20	0.50*	0.068	0.083
Robertson	4504	7639	15	0.30	0.057	0.080
Round	4430	7753	20	0.62	0.261	0.348
Scott	4808	8115	11	0.22	0.418	0.566
Scugog	4410	7850	20	0.61*	0.078	0.138
Seymour	4423	7749	25	0.60*	0.108	0.142
Sharbot	4446	7641	25	0.05	0.190	0.220
Simcoe	4425	7925	22	0.72*	0.142	0.213
St. John	4441	7919	20	0.51*	0.106	0.267
Stony	4433	7806	20	0.83	0.129	0.199
Sydenham	4425	7633	5	0.58	0.391	0.536
Temperance	4436	7553	20	0.16	0.228	0.374
Theriault	4640	8018	16	0.30	0.074	0.133
Timberwolf	4541	7848	18	0.64	0.390	0.588
Urbach	4450	7749	20	0.72	0.240	0.479
Vermilion	4630	8126	10	0.12	0.163	0.189
Wawiashkashi	4648	8020	8	0.87	0.123	0.293
White Partridge	4550	7806	7	0.60	0.233	0.348
Whitewater	4911	8011	15	0.04	0.377	0.440
Wicksteed	4646	7940	4	0.84	0.263	0.346
Wilson	4844	8049	10	0.78	0.125	0.333
Wolverine	4950	8346	8	0.73*	0.062	0.139

Table 2. Summary statistics for standardized [Hg] (ppm wet weight) in dorsal muscle tissues of yellow perch in 57 Ontario lakes.

	[Hg] 20 cm perch	[Hg] 25 cm perch
Minimum	0.039	0.080
Maximum	0.505	0.765
Mean	0.194	0.302
Standard deviation	0.110	0.161
# lakes [Hg] >0.30 ppm	8 (14%)	24 (42%)

Coefficient of determination (R^2) values ranged from 0.04 - 0.97, with an average value of 0.53 (Table 1). However, it should be noted that in lakes where correlations between fish length and [Hg] are poor, estimated standard length [Hg] may simply approximate the average mercury concentrations in prey of all sizes.

The buffering capacities of the lake catchments were negatively correlated with [Hg] in both 20 cm and 25 cm perch ($R^2 = 0.16$ and 0.14 respectively). These correlations were statistically significant at $p < 0.005$. The overall geographical distribution of [Hg] in yellow perch in the 57 lakes appeared random, and there were no evident spatial trends. Most of the lakes sampled are located in southeastern Ontario, including the Haliburton, Muskoka, and Georgian Bay regions (Table 1).

Discussion

Fimreite [11] proposed that the extremely poor reproductive success he observed of common loons on some lakes of the Wabigoon-English River system was due to mercury contamination. This hypothesis was later confirmed in a more detailed study by Barr [2], in which he found a strong inverse correlation between the breeding success of loons and the levels of mercury contamination in lakes in the Wabigoon system. The results of the present study indicate that there are a considerable number of Ontario lakes in which mercury levels in yellow perch are high enough to pose a threat to common loon reproduction. Although none of the lakes included here are known by the author to have point sources of mercury contamination, 14 percent of the lakes are estimated to have 20 cm yellow perch with [Hg] > 0.30 ppm. This proportion increases to 42 percent of the lakes for 25 cm yellow perch (Table 2). In general, 85 percent to 95 percent of the total mercury in these fish will likely be in the methylated (and therfore most bioaccumulative and toxic) form [12].

It is expected that this random sample of lakes is representative of the mercury levels in yellow perch throughout much of Ontario, as the range in perch [Hg] reported here is similar to that previously recorded in uncontaminated Ontario lakes [2]. Exposure to mercury for loons inhabiting areas impacted by point source discharges of mercury should be considerably higher. One such example is Clay Lake, Ontario, which previously received direct discharges from a chlor-alkali plant. Yellow perch sampled in Clay Lake averaging only 16.6 cm had a mean [Hg] of 1.20 ppm [2].

The results reported here show that the potential for dietary exposure to mercury at levels harmful to common loon reproduction is widespread in Ontario, and support the hypothesis that the entire North American population may be affected. However, it must be stressed that the diets of loons are expected to vary considerably in both the size and species of their prey throughout the study area, and that this variability may have a profound impact on their exposure to mercury. Nonetheless, it is expected that the mercury levels of other loon prey should be similar to those of perch within specific territories. For example, Barr [2] observed that [Hg] in similar size pike (*Esox lucius*), pickerel (*Esox americanus*), sauger (*Stizostedion canadense*), and mooneye (*Hiodon tergisus*) are generally higher than in yellow perch, while concentrations in cisco (*Coregonus arterdi*), whitefish (*Coregonus clupeaformis*) and white sucker (*Catostromus commersoni*) may be equal to or lower those of similar size perch. Similarly, Parks [12] found that the [Hg] in pike and crayfish were positively correlated with those of yellow perch, across a wide range of environmental mercury levels.

Given the underlying relationship between fish length and [Hg] (Figure 1), it was expected that the distribution of yellow perch [Hg] would be related to the mean size of the fish sampled in each lake. However, no significant relationship was observed between either the average length or the average weight of all fish sampled in each lake, and the mean [Hg] of those fish.

The only significant correlation ($p < 0.005$) found with standardized perch [Hg] was the buffering capacity of the lake catchments. Approximately 15 percent of the variation in [Hg] of both 20 and 25 cm yellow perch was explained by the catchment type, and more poorly buffered drainage basins were associated with higher [Hg] in perch. This finding is in agreement with numerous studies that have shown that concentrations of mercury in aquatic biota are correlated with lake acidity. Lower pH is known to enhance the formation of methylmercury, the predominant form of mercury that accumulates in fish tissues [13]. The inverse relationship between lake pH and fish mercury contamination is well documented [14-16].

In addition to the mobilizing effects that acidity is known to have on catchment mercury, elevated inputs of acid deposition and airborne mercury are likely to occur together [17]. Most of the mercury entering Ontario lakes is of anthropogenic origin, being deposited from the atmosphere, although in some catchments a significant amount may be geologically derived [18,19]. Therefore, it is expected that the prey mercury levels reported here are representative of those encountered in regions of similar geologies, and atmospheric acid deposition levels, throughout much of the summer range of the common loon in North America.

It is a logical extension of our current understanding of mercury contamination in aquatic systems to assume that fish-eating birds feeding in low-pH lakes will have relatively higher dietary exposure to mercury. It is recommended that future studies of mercury contamination in common loons focus on acidic lakes, where mercury-related effects on populations are most probable to be manifest. Recent studies have found that brood mortalities are highest, and loon production tends to be lowest, on low-pH lakes in Ontario [20,21]. Although these trends have been attributed to reduced food availability in acid lakes [20], undoubtedly the potential for the synergistic effects of low pH and elevated Hg exists. However, it must be noted that, in addition to exposure to mercury at chronically toxic levels, there are several factors that are likely to contribute to the decline of common loon populations in North America. These include the loss of breeding and rearing habitat (through human encroachment and hydroelectric development), reductions in prey (notably through the effects of lake acidification), and exposure to a suite of contaminants discharged into the environment through various anthropogenic activities.

It is hoped that this study may be used as a directive tool for future work aimed at determining the extent and effects of Hg levels in prey on the behavior, reproductive success, and survival of common loons, along a gradient of environmental exposure. The determination of contaminant levels in prey is a useful step in the identification of areas where the potential for toxic effects on loons (and other fish-eating birds) exists, without requiring the disturbance or sacrifice of any birds. However, future research involving direct measurement of mercury levels in common loons is essential to adequately assess the extent and effects of mercury contamination.

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DETERMINANTS OF THE SHORT-TERM DYNAMICS OF PCB UPTAKE BY PLANKTON.

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Abstract:

Many equations show that various aspects of the ecological fate of a contaminant are partly determined by chemical structure, but few studies combine such "quantitative structure-activity relations" with environmental properties that may modulate those effects, so the importance of such properties remains unquantified. This study determines the effects of variations in suspended biomass, dissolved and colloidal organic carbon, and pH on the time course of 2,2',4,4',5,5'-hexachlorobiphenyl uptake by laboratory cultures of Selenastrum capricornutum. Variations in pH had no effect, but uptake was enhanced by higher levels of biomass (in ppm by volume) and depressed by higher levels of organic carbon (Abs, m^{-1}, measured as absorbance at 440 nm). The laboratory coefficients for these measured effects on hexachlorobiphenyl were combined with existing relations based on molecular connectivity (X) or capacity ratio (K') to yield semi-empirical equations to predict the instantaneous rate of uptake (rate, $\% \text{ min}^{-1}$) and bioconcentration factor (BCF) of organic contaminants as:

$$\text{Log rate} = -3.30 + 0.32\ X + 1.1\ \text{Log biomass} - 0.42\ \text{Log Abs}$$

$$\text{Lof BCF} = 4.11 + 0.86\ \text{Log}\ K' - 0.87\ \text{Log biomass} - 0.22\ \text{Log Abs}$$

The utility of these equations was assessed by comparing time courses of hexachlorobiphenyl uptake predicted from them with time courses observed in water from eleven Quebec lakes. Plots of observed vs predicted values from three

replicate runs for each lake had a mean coefficient of determination (r^2) of 0.84(range = 0.64 to 0.95); the average slope (0.84) did not differ from unity (range = 0.49 to 1.75) nor did the average intercept (2.1) differ from zero (range = -6.2 to 11.2). Similar predictions which ignore the effects of color and biomass had similar r^2 values (mean = 0.8, range = 0.51 to 0.93) in plots of observed vs predicted uptake, but the higher slopes (mean = 2.80, range = 1.52 to 3.73) and lower intercepts (mean = -6.7, range = -19 to 6.5) indicated that the prediction based only on contaminant properties underestimated observed uptake.

Résumé

Plusieurs équations démontrent que de nombreux aspects de l'impact environnemental d'un contaminant sont partiellement déterminés par la structure chimique de ce dernier. Seules quelques études combinent les relations quantitatives entre la structure chimique d'un contaminant et ses activités biologiques, avec les propriétés environnementales qui peuvent altérer ces effets. L'importance de ces propriétés demeure donc trop souvent ignorée. Cette étude évalue les effets occasionnés par une variation de la biomasse en suspension, du carbone organique dissout et colloidal, et du pH sur la course temporelle d'assimilation d'hexachlorobiphényl-2,2',4,4',5,5' par une culture de l'algue verte Selenastrum capricornutum. La variation du pH n'a eu aucun effet, cependant, le taux d'assimilation a été rehaussé par les concentrations élevées en biomasse (en ppm par volume) et réduit par les concentrations élevées en carbone organique (Abs, m^{-1}, mesurée comme étant l'absorbance à 440 nm). Les coefficients expérimentaux de ces effets ont été combinés aux relations existantes basées sur la connectivité moléculaire (X) ou le facteur de capacité (K'). Des équations semi-empiriques ont été crées pouvant prédire le taux

d'assimilation (rate, % min^{-1}) et le facteur de bioconcentration (BCF) des contaminants organiques;

$$\text{Log rate} = -3.30 + 0.32\,X + 1.1\,\text{Log biomasse} - 0.42\,\text{Log Abs}$$

$$\text{Log BCF} = 4.11 + 0.86\,\text{Log }K' - 0.87\,\text{Log biomasse} - 0.22\,\text{Log Abs}$$

L'utilité de ces équations fut évaluée en comparant les courses temporelles d'assimilation prédites par celles-ci avec les courses temporelles observées en utilisant l'eau de onze lacs du Québec. Les graphiques représentant les valeur observées en fonction des valeurs prédites, issues de trois réplicats effectués en laboratoire pour chaque lac, avaient un coefficient de détermination moyen (r^2) de 0.84 (variation = 0.64 à 0.95), une pente moyenne (0.84) non différente de l'unité (variation = 0.49 à 1.75) et un abcisse à l'origine moyen (2.1) non différent de zéro (variation = -6.2 à 11.2).

Les graphiques représentant les valeurs observées en fonction des valeurs prédites utilisant les équations qui ignorent les effets de la couleur et de la biomasse avaient des valeurs de r^2 similaires (moyenne = 0.8, variation = 0.51 à 0.93) cependant leur pente était plus élevée (moyenne = 2.80, variation = 1.52 à 3.73) et leur valeur d'abcisse à l'origine inférieure (moyenne = -6.7, variation = -19 à 6.5). Ces résultats indiquent que les prédictions basées uniquement sur les propriétés du contaminant à l'étude sous-estime la course temporelle d'assimilation observée.

THE ROLE OF SEDIMENT AND FOOD WEB STRUCTURE IN THE DISTRIBUTION OF PCB CONGENERS IN SMALL LAKES. C.R. Macdonald, and C.D. Metcalfe, Environment Canada, Canadian Wildlife Service, Hull, Québec, and Environmental and Resources Studies Program, Trent University, Peterborough, Ontario.

Food web structure and adsorption of PCBs onto sediments are two factors which have significant influence on the type and amount of PCB accumulated in the upper trophic levels in small lakes. This hypothesis was tested by sampling several groups of biota, suspended sediments and bottom sediments in 7 small lakes which contained PCBs from either point-sources or the atmosphere. Biota/sediment and biota/zooplankton ratios were used to indicate the relative distribution of 19 congeners in the lakes. Biota (lip wt)/ sediment (org C basis) ratios of zooplankton and perch ranged from 1 to 10 and 4 to 30 respectively, and showed a negative correlation between maximum lake depth and the biota/zooplankton ratios for bass and perch. Principal component analysis indicated that there were also major species differences in the relative amounts of the individual congeners accumulated in any lake. These data suggest that a complex set of factors including lake morphometry, sediment transport and food web structure influence the amount and relative abundance of PCB congeners in the upper trophic levels of any specific lake.

WHY DON'T GREAT LAKES FISH REFLECT ENVIRONMENTAL LEVELS OF ORGANIC CONTAMINANTS? D.F. Rowan and J.B. Rasmussen, Environmental Research Laboratory, Chalk River Nuclear Laboratories, Chalk River, Ontario, and Department of Biology, McGill, Montréal, Québec.

We have reviewed the literature on two persistent organochlorines (PCBs and DDT) in the Great Lakes ecosystem, in an attempt to explain between-basin and between-species variation in fish contamination. Empirical models were developed using log-linear multiple regression linking tissue contaminant levels (water and sediments) as well as basin-specific ecological attributies. Levels of PCBs and DDT in sediment and water can explain between-basin variability in fish contaminant levels only when basin-specific ecological attributes are taken into account. The important factors that appear to determine the ecological partitioning of persistent organic contaminants are fish lipid content, trophic position, and the trophic structure of the food chain. These multiple regressions explained 59% (DDT) to 72% (PCBs) of the variation in contaminant levels of 25 species of Great Lakes fish. We suggest that sustained piscivore yield relative to primary productivity is indicative of food chain length, and point to the need for more direct quantification of trophic relationships in the Great Lakes.

BIOACCUMULATION OF PCBs IN LAKE ONTARIO FISH. E. Bentzen, D.R.S. Lean, C. Hauschield and W.A. Scheider, Trent University, Peterborough, Ontario, and Environment Canada, National Water Research Institute, Burlington, Ontario, and Ontario Ministry of the Environment, Toronto, Ontario.

Total polychlorinated biphenyls (PCBs) in lake trout fillets were found to be a function of planktivory, percent lipid and lattitude (Rasmussen et al. 1990). This relationship was developed from the Ontario Ministry of the Environment (OMOE) sport fish contaminant monitoring program and used lake means of PCB measured in skinless and boneless dorsal fillets. The model was further evaluated for additional OMOE lakes on individual trout and other fish species. Considerable within lake variability of PCB in lake trout of a given size is demonstrated. The model was applied to other fish species such as smallmouth bass and walleye. Although changes in trophic structure due to the presence of planktivorous fish and freshwater shrimp (Mysis relicta) were important in the original predictive model, multicolinearity occurs with the other independent predictors (percent lipid and latitude).

LIPID CONTAMINANT INTERACTIONS. B.C. Wainman and D.R.S. Lean, Department of Biology, University of Waterloo, Waterloo, Ontario, and Environment Canada, National Water Research Institute, Burlington, Ontario.

The lipids which sequester contaminants are often neglected when considering the dynamics of liphophilic contaminants. Most contaminant models, and contaminant modellers, consider lipids to be unchanging components in the water column. The validity of this assumption was tested in a comprehensive study of the lipids in planktonic organisms from three headwatej lakes of varying trophy near the NWRI field station near Peterborough, ON. For zooplankton it was found that the lipid fraction of dry weight and the lipid species varied markedly over the season while in phytoplankton the lipid fraction varied somewhat less but appeared related to water temperature and daylength. Given that zooplankton and phytoplankton biomass vary greatly over the season, planktonic lipids vary immensely between seasons. Lipids are, it appears, as diverse and dynamic as the contaminants they partition.

SESSION 2A

APPROACHES TO EVALUATING THE ECOLOGICAL RELEVANCE OF BIOMARKERS OF STRESS/MÉTHODES D'ÉVALUATION DE LA PERTINENCE, SUR LE PLAN ÉCOLOGIQUE, DES BIOINDICATEURS DES MARQUEURS BIOLOGIQUES DE STRESS

CHAIRPERSON/PRÉSIDENTE
Diane Malley

BEHAVIORAL BIOMARKERS INDICATIVE OF STRESS IN Daphnia. D.C. McNaught and D.C. Drake, Department of Ecology, Evolution and Behavior, University of Minnesota, Minneapolis, Minnesota.

INTRODUCTION

Behavioral modifications have resulted from chronic exposure to toxic contaminants introduced into Great Lakes ecosystems. Such modifications threaten ecosystem integrity. Behavioral changes have been documented in the herring gull, as well as in infants of human mothers, with fish consumption involved in both cases. Yet ecotoxicologists have just begun to perform such assays with zooplankton which have the advantages of ease of culture, short generation times, and high fecundity. We have recently shown that NaBr modifies the response of *Daphnia* to the angular light distribution, i.e., NaBr reduces their ability to discern contrast. In this paper, we present two new behavioral markers.

Behavioral responses to light comprise the exogenous component of vertical migrations undertaken by zooplankton. Here two behaviors, the well known swimming behavior under planar polarized light, and the less understood tremor of the compound eye, both in *Daphnia*, are examined for modifications by a chemical which commonly pollutes lakes. Thus, these behaviors are potential biomarkers of stress.

MATERIALS AND METHODS

To examine responses to polarized light, and their modification by NaBr, two animals were placed in a 5cm petri dish containing lakewater (control and polarized control) and as well in lakewater with 10^{-5}m, 10^{-3}m, and 10^{-1}m NaBr. Animals were allowed to acclimate for 10 min. They were then placed on the bed of an overhead projector, and their swimming paths traced onto paper attached to a wall. The animals' positions were noted every three seconds. After making such tracings for ten animals at each of three concentrations of NaBr, and with white light and polarized light controls, the angles of change in direction were measured by hand. Nine angles for each tract of 10 animals were entered into a program to calculate Raleighs′z. These were cast into a one-way ANOVA (Statistic, copyright) to determine the significance of treatment.

To measure eye tremor, animals were placed into 5cm petri dishes. After acclimation for 10 minutes in these same control and experimental conditions, the oscillations of the compound eye about the central nervous system were counted. The movements (oscillations per minute) were cast into a one-way anova again to determine the significance of treatment.

RESULTS

Responses to polarized light under control conditions consisted of movements perpendicular to the *e*-vector, a response known for 40 years. The presence of very low concentrations of NaBr caused these *Daphnia* to swim in circles. The higher concentrations of NaBr led to smaller, tighter circles. The ANOVA run on the resulting Raleighs' zs indicated that the effects of NaBr concentration were significant ($P<0.1$).

Tremor in the compound eye was influenced by the concentration of NaBr. The rate of oscillation was reduced four-fold by 0.1 m NaBr. Using a one-way ANOVA, we showed that the effects of NaBr on tremor were highly significant ($p<0.00$).

DISCUSSON

These two behavioral biomarkers were sensitive to a halogenated compound, NaBr. This is likely because the bromide ions interferred with the chloride channels associated with nerve transmission.

The polarized light assay is difficult to run. Employment of new techniques (frame grabber coupled to personal computer) will make calculation of Raleigh's z easy and fast. The EC_{50} is more than 2x lower than for impairment of reproduction in *Daphnia*. It can become a widely used behavioral biomarker.

The tremor assay is easy to run. The EC_{50} is equivalent to the LC_{50} for NaBr. It will not likely gain acceptance.

SIGNIFICANCE OF LIVER NEOPLASIA IN WILD FISH: ASSESSMENT OF PATHOPHYSIOLOGIC RESPONSES OF A BIOMONITOR SPECIES TO MULTIPLE STRESS FACTORS.

G.M. Kirby and M.A. Hayes, Department of Pathology, University of Guelph, Guelph, Ontario.

ABSTRACT

Diseases of wild fish can be used to monitor the quality of the aquatic environment. However, a detailed understanding of the pathogenesis of the diseases being monitered is necessary for the accurate evaluation of epidemiologic studies used in the assessment of water quality and the status of the aquatic environment. Fish and terrestrial animals have developed complex defense systems that provide resistance to a variety of environmental stresses. For example, glutathione transferases (GSTs), a multifunctional, multigene family of enzymes are involved in the detoxification of various environmental carcinogens including polycyclic aromatic hydrocarbons (PAHs) to which wild fish are exposed. The hepatic cytosolic GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) and the protection provided by cytosolic GSTs against binding of the reactive PAH metabolite benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE) to hepatic DNA was examined in various benthic and pelagic species of fish. GSTs in bottom-dwelling species such as brown bullhead, European carp, white sucker and channel catfish have greater CDNB conjugating activity than pelagic species including trout, perch, and salmon. This variability in GST activity in benthic fish corresponds with greater GST-mediated protection against DNA binding by BPDE suggesting that benthic fish are quite resistant to BaP, and possibly other environmental carcinogens, due to constitutive GST-related detoxification systems.

Susceptibility of fish to PAHs might also be influenced by acquired alterations in GST detoxification capabilities. A parasite-associated inflammatory bile duct disease is frequently observed concurrently with liver neoplasia in white suckers from polluted areas of Lake Ontario. This chronic inflammatory bile duct disease, characterized by cholangiohepatitis and cholangiofibrosis, frequently results in segmental obstruction of liver lobes. We have examined the influence of this parasitic hepatobiliary disease on hepatic GST activity and benzo(a)pyrene metabolism and genotoxicity. Cytosolic glutathione S-transferase (GST) activity (CDNB) was significantly reduced in obstructed liver when compared with adjacent grossly unobstructed liver. Preincubation with µmolar concentrations of endogenous nonsubstrate ligands such as CDCA, deoxycholic acid, bilirubin and hematin resulted in a dose-related inhibition of cytosolic and S-hexylglutathione-affinity-purified GST activity (CDNB conjugation) *in vitro*. Bilirubin also inhibited the activity of hepatic cytosolic GSTs towards benzo(a)pyrene-4,5-oxide. Cytosol from adjacent liver had only a moderately stronger protective activity against DNA binding by BPDE than did cytosol from obstructed liver. Suckers with segmentally obstructed livers, identified by exploratory laparotomy, were orally administered ^{3}H-benzo(a)pyrene (^{3}H-BaP) (0.2 µmol/kg) and the level of BaP-macromolecular binding was analyzed in obstructed and adjacent unobstructed liver tissue. Covalent binding of ^{3}H-BaP to hepatic protein was 30% less in adjacent unobstructed liver compared to obstructed liver, however, there was no significant difference in the level of BaP bound to DNA in the two regions. These studies indicate that parasitic liver disease and associated bile duct obstruction and some of the endogenous nonsubstrate ligands that increased after obstruction, are associated with reduced hepatic GST activity. This suggests that resistance to some unidentified environmental carcinogens that are necessarily detoxified by GSTs might be compromized in fish with concurrent parasitic liver disease. The concept of constitutive and acquired variations in resistance to environmental carcinogens in fish bioindicator species has important implications in the evaluation of water quality and potential risks to humans.

INTRODUCTION

The quality of the aquatic environment can be monitored by the epidemiological assessment of diseases of wild fish (1, 2, 3). For example, the prevalence of liver neoplasia in various species of bottom-dwelling fish is currently used to assess the status of both marine and freshwater environments (4, 5, 6) However, an understanding of the various factors influencing the development of these pollution-associated liver neoplasms has important implications for efforts to monitor environmental carcinogens by tumor surveys in fish. Chronic degenerative diseases, such as cancer, result from a deterioration or deficiency in host resistance to carcinogens. Variations in constitutive and acquired resistance mechanisms within and between fish species might explain differences in susceptibility to environmental carcinogens such as polycyclic aromatic hydrocarbons (PAHs). We are currently investigating the factors that might influence the pathogenesis of liver neoplasia in various bottom-dwelling species from the Great Lakes particularly white suckers (*Catostomus commersoni*) from industrially-polluted western Lake Ontario (4, 7, 8). Information derived from studying liver tumors in these fish is valuable not only in terms of increasing our knowledge of the factors involved in the pathogenesis of naturally-occurring liver cancer in fish, but also by providing information on the biological effects of anthropogenic alterations to the aquatic environment. The results of this study suggest that multiple factors including variability in the activity and expression of PAH-metabolizing enzymes, particularly glutathione S-transferases (GSTs), and concurrent inflammatory hepatobiliary disease might influence the resistance to environmental carcinogens in fish.

MATERIALS AND METHODS

Reagents. ^{3}H-Benzo(a)pyrene (^{3}H-BaP, specific activity 56 Ci/mmol) was a product of New England Nuclear (Boston, MA). (±)-r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydro [1,3-^{3}H] benzo[a]pyrene (anti) (^{3}H-BPDE, specific activity 439 Ci/mmol) was obtained from Chemsyn Science Laboratories, Lenexa, KS. (±) Benzo(a)pyrene-4,5-dihydroepoxide (BaP-4,5-oxide) was purchased from the NCI Chemical Carcinogen Repository, (Kansas City, MO). Chenodeoxycholic acid (99% purity), deoxycholic acid, bilirubin, biliverdin, hematin, GSH, sodium dodecyl sulfate (SDS),1-chloro-2,4-dinitrobenzene (CDNB), 2-phenoxyethanol and calf thymus DNA (type 1) were purchased from Sigma Chemical Co. (St. Louis, MO.). NADPH, ribonuclease (RNase A) and protease (Proteinase K) were products of Boehringer-Mannheim Biochemicals (Dorval, Quebec, Canada). Methanol (HPLC grade) and trichloroacetic acid were obtained from Fisher Scientific Co. (Nepean, Ontario, Canada). Hydroxyapatite (DNA grade, Bio-Gel HTP) was obtained brom Bio-Rad Laboratories (Mississauga, Ontario, Canada). All other chemicals were of reagent grade.

Animals and Treatments. Liver samples from brown bullhead, carp, catfish, sculpin, and perch were generously provided by I. Smith, Ontarion Ministry of the Environment, Rexdale, Ontario, and liver from trout and salmon were obtained from H. Ferguson, Department of Pathology, University of Guelph, Guelph, Ontario. Adult male white suckers (n=241) were caught from the Humber River (Toronto, Ontario, Canada) during their spring spawning migrations and were maintained for 1 week in laboratory holding tanks in clean well water at 10°C. Laparotomies were performed on fish under anesthetic (2-phenoxyethanol, 5ml/liter water) in order to detect segmental liver obstructions. These obstructed regions occurred consistently in the most distal liver lobes and were easily identified because they were stained a dark brown-green color due to retained bile pigments. The existence of segmentally obstructed liver lobes in these fish provides a unique opportunity to compare the metabolism and genotoxicity of carcinogens in areas of liver affected, to a greater or lesser degree, by cholestasis. For studies on the binding of BaP metabolites to liver macromolecules *in vivo*, fish with obstructions were orally administered ^{3}H-BaP (0.2 µmol/kg of body weight; 2 ml of corn oil/kg of body weight) 1-2 days after surgery. Fish were killed 24 hours later and samples of obstructed and adjacent unobstructed liver were separated and frozen for adduct analysis. Necropsies were performed on other fish and liver tissue from those with segmental obstructions was used to prepare cytosol for analysis of GST activity and for the extraction of bile acids. Cytosol was also prepared from the liver of fish without gross evidence of obstructive bile duct disease for use in enzyme inhibition assays and in the affinity purification of GST.

Histopathology and Isolation of Parasites. This study did not focus on the prevalence or histopathological characteristics of hepatocellular or cholangiocellular neoplasms in white suckers as this topic has already been covered in other studies (4, 7, 9). Histopathological studies were performed on white sucker liver in order to assess the prevalence of inflammatory liver disease and parasite infestation in fish from polluted and reference sites in the Great Lakes. Histological sections of liver from white suckers from in Lake Ontario (Humber R., N=46 and Ganaraska R., n=40) and Lake Huron (South Bay, N=47 and Spanish R., N=46) were generously provided by Dr V. Cairns, Canadian Center for Inland Waters, Burlington, Ontario, Canada. Liver tissue was fixed in 10% formalin and 5μm paraffin sections were stained with hematoxylin and eosin (H&E). Sections were examined for the presence of inflammatory lesions which were broadly categorized as either cholangiohepatitis or cholangiofibrosis by criteria described in the results. The lesions were graded on a scale of 0 to 4 (0: no visible lesions; 1: mild; 2: moderate; 3: marked; 4: severe) according to the severity of the lesions. Evidence of parasitism was assessed by quantifying the number of parasites or parasitic granulomas visible per section. The location of parasites (i.e. within bile ducts, blood vessels or hepatic parenchyma) and evidence of parasite-associated inflammation (ie migratory tracts) was also noted. Parasites were also isolated from liver tissue by manual extraction under a dissecting microscope or by pepsin digestion using a Baerman's apparatus.

Preparation of Hepatic Cytosols and GSTs. Liver tissue from the various species of fish and from obstructed and adjacent unobstructed liver from white suckers with gross evidence of obstructive bile duct disease were collected on ice, minced, and homogenized in 3 volumes of 0.25 M sucrose (pH 7.4), at 4°C, containing 1 mM EDTA and 25 mM Hepes. Cytosol was prepared as the final supernatant of centrifugation at 10,000g (30 min, 4°C) and 100,000 g (60 min 4°C) (10). Cytosol, from the livers of suckers unaffected by obstructive bile duct disease, used for GST purification by affinity gel chromatography, was dialyzed for 18 hr (12-14 kDa cutoff) against 10 mM Tris-HCl containing 50 mM NaCl (pH 7.8, at 4°C) and applied to a column (1X20 cm) containing *S*-hexylglutathione-Sepharose 4B (Pharmacia, Etobicoke, Ontario). Following extensive washing with 200 mM NaCl, GSTs were eluted with the wash buffer containing 5 mM *S*-hexylglutathione (10). The fractions exhibiting GST activity by 1-chloro-2,4-dinitrobenzene (CDNB) conjugation were pooled, dialyzed for 24 hr against 10mM Tris-HCL (pH 7,5) and stored at -70°C. The affinity-purified GST preparations contained only bands in the 24-28 kD range present in silver-stained polyacrylamide electrophoresis gels (Fig. III.9.), the purity of which was confirmed by western immunoblot analysis (7).

Enzyme Assays and Inhibition Studies. The activity of cytosolic GST and affinity purified GST towards the substrates CDNB and BaP-4,5-oxide was determined. GST activities using CDNB (0.5 mM, pH 6.5) as a substrate were measured at 25°C by the spectrophotometric method of Habig *et al*., (11). Specific activities were expressed as μmol CDNB/min/mg protein. The assay of GST activity towards BaP-4,5-oxide was based on a method described by Eaton and Stapleton (12). The effect of chenodeoxycholate, deoxycholate, bilirubin, and hematin on the activity of sucker hepatic GSTs towards CDNB was measured by comparing the reaction rate in the absence and the presence of the inhibitors. The effect of bilirubin, hematin and CDCA on cytosolic GST activity using BaP-4,5-oxide as a substrate was also determined. The enzyme (25 μl containing 40 μg cytosolic protein or 2.5 μg of affinity purified GST) was preincubated with the inhibitor for 5 minutes prior to the addition of substrates. In the CDNB assays, at least five different concentrations of inhibitors, in the μmolar range, were used in the assays and the I_{50} values (the concentration giving 50% inhibition of enzyme activity) were determined from plots of remaining activity vs inhibitor concentration. Because of the limited solubility of CDCA, 2.0% (v/v) methanol was included in the assay for this bile acid. Hematin, biliverdin and bilirubin were dissolved in 10 mM NaOH and all solutions were protected from light. The volume of the additions of inhibitor solutions was 20 μl. Control experiments were performed in the absence of nonsubstrate ligand but included appropriate concentrations of methanol or NaOH carrier solutions. Activities represent the average of 3 determinations.

Separation of GSTs on Polyacrylamide Gels. Polypeptide subunit profiles of cytosol and purified GSTs were analyzed by SDS-polyacrylamide (12%) gel electrophoresis (SDS-PAGE) as described by Rushmore et al., (10). All gels were stained with silver nitrate following the precedure described by Wray et al. (13). Densitometric analysis of polypeptide bands in the 26 kD molecular weight region was performed by scanning the gels on an Ultrascan XL Enhanced Laser Densitometer (LKB, Bromma, Sweden).

DNA Binding Assays. The DNA protective activities of cytosols from livers from various species of freshwater fish including brown bullhead, carp, catfish, sculpin, trout, perch as well as obstructed and adjacent unobstructed and affinity purified GSTs from white sucker liver were assayed in an *in vitro* DNA binding procedure similar to that described by Quinn et al. (14). Each 1-ml reaction mixture contained 0.1 M K-phosphate buffer (pH 7.4), 0.1 M sucrose, 0.5 µCi ^{3}H-BPDE (1 µM) dissolved in 20 µl of tetrahydrofuran/triethylamine (19:1), 0.2 mg calf thymus DNA, NADPH (2mM), 1.6 mg cytosolic protein (i.e. cytosol from obstructed or adjacent unobstructed liver) and various concentrations of dialyzed affinity-purified liver GSTs with or without GSH (2.5 mM). Samples were incubated for 8 min at 25°C, and then extracted three times with chloroform/isoamyl alcohol (10:2, v/v). Ethanol-precipitated DNA was collected by centrifugation, washed with ethanol, and dissolved in 1 ml 10 mM Tris-HCl/1 mM EDTA (TE buffer, pH 7.4). DNA was purified by incubation with ribonuclease A and proteinase K, and then extracted, precipitated and redissolved as described above. The DNA in solution was quantified by 260 nm absorbance, radioactivity was measured by LSC using a Beckman LS-3133 spectrometer and ^{3}H-BPDE bound to DNA was expressed as pmol bound/mg DNA.

BaP metabolites bound to sucker hepatic DNA *in vivo* were also measured. BaP-modified DNA was isolated from normal and obstructed liver tissue by hydroxylapatite chromatography according to the method described by Adriaenssens et al. (15). The DNA was quantified by 260 nm absorbance using the relationship 1mg DNA=50 A_{260} units. The purity of the final preparation was judged by the absorbance ratio of A260 nm:280 nm which consistently ranged from 1.8-2.0. BaP-derived radioactivity was then assessed in the DNA solution by direct liquid scintillation counting (LSC) and the specific binding of BaP to DNA was expressed as fmol BaP/mg DNA.

Protein Isolation and Quantification of BaP Covalently Bound to Protein. Protein was isolated from the CIP extract obtained during the isolation of DNA as described by Stowers and Anderson (16). BaP-derived radioactivity in the protein solution was then assessed by LSC. The covalent binding of BaP to protein was expressed as fmol BaP/mg protein.

RESULTS

Gross and Histologic Lesions. Many white suckers had a diffuse inflammatory proliferative reaction along portal tracts, which is subsequently referred to as cholangiohepatitis. Liver affected with cholangiohepatitis had diffuse lesions that were consistently centered on major bile duct systems. Major portal tracts were prominent and tortuous with markedly hyperplastic biliary epithelium frequently folded into papillary lumenal projections. Duct lumens often contained protein- and/or mucus-rich material together with exfoliated necrotic epithelial cells and some leukocytes. Surrounding these biliary tracts was an associated marked chronic periductal inflammatory reaction characterized by a mixed mononuclear cell infiltrate together with a mild to severe degree of periportal fibrosis. There was occasionally periportal proliferation of small cholangioles that extended into the hepatic parenchyma.

A less frequent but distinct process, subsequently termed cholangiofibrosis, occurred mainly as relatively discrete focal lesions in the peripheral regions of the liver; the long narrow distal lobes being most frequently affected. Some of these distal obstructed lobes were dark green segments that were readily distinguished from the tan colored proximal adjacent unobstructed liver. The more chronic and fibrotic lesions appeared as discrete pale foci (2-20 mm) present within obstructed lobes. Histologically, affected areas had focal segmental proliferations of small cholangioles, accompanied by moderate to severe interductal fibroplasia . In the more severely affected regions, there was marked hepatocellular atrophy with few residual hepatocytes recognizable among the cholangiofibrotic reaction. Melano-macrophage centers comprised of greatly enlarged phagocytes containing yellow brown cytoplasmic pigment were prominent in these areas. Leukocytic infiltrates were minimal except in the vicinity of migratory parasites, encapsulated granulomas or concurrent areas of cholangiohepatitis. The distribution and histologic appearance of the cholangiofibrotic lesions was consistent with a segmental bile duct obstruction.

Several different parasites were observed both grossly and histologically in the livers of these fish. Protozoal cysts (*Myxozoa: Myxosporea*) were visible within major bile ducts the epithelium of which was hyperplastic. Nematode larvae (*Spirurida: Acuarioidea*) were located within acutely necrotic areas of hepatic parenchyma and were associated with infiltrates of granulocytic and mononuclear inflammatory cells or were present within encapsulating granulomata. Flukes *(Diginea: Sanguinicola)* were present mostly in portal veins but occasionally within areas of acutely necrotic tracts in the hepatic parenchyma .

Hepatocellular neoplastic lesions ranged from small round or irregular foci of phenotypically altered (basophilic) hepatocytes to larger hepatocellular neoplasms that were expansive or often locally invasive within the liver. These lesions were composed of polygonal cells with a higher nucleus to cytoplasmic ratio and less cytoplasmic differentiation than normal hepatocytes. Lesions corresponding to foci of hepatocellular alteration, were occasionally visible grossly as small (2-10 mm) dark brown spherical areas scattered throughout the liver. Biliary neoplasms consisted of either bile duct adenomas, typically discrete nodules of irregularly arranged ducts within a connective tissue matrix, or carcinomas, typified by extensively invasive, poorly differentiated, irregular duct-like structures.

The prevalence of inflammatory bile duct disease in white suckers was determined by histological examination of sections of white sucker livers from two polluted and two reference sites in the Great Lakes. Quantification of mean histological scores reflecting the severity of lesions of cholangiohepatitis and cholangiofibrosis revealed that inflammatory bile duct disease is widespread in white suckers throughout the Great Lakes (Fig.1). While some livers from all sites had evidence of Sanguinicola, Myxosporea, and Spirurid parasitic infestations, there were no obvious differences in the frequency of infestations among types of parasites and sites.

Assays for GST Activity and Expression. The hepatic cytosolic GST activities (CDNB) from various species fish including brown bulhead, white sucker, catfish, sculpin, perch, trout and salmon are presented in Fig. 2. Hepatic cytosolic GST activity varied significantly in the various species examined . Generally, the GST activity was greater in benthic fish species compared to pelagic fish. The influence of bile duct disease and cholestasis on cytosolic GST activity was also determined. GST activity (CDNB conjugation) of cytosol prepared from obstructed and adjacent unobstructed liver was examined. GST activity in cytosol prepared from obstructed liver (mean ± SE, 0.25 ±0.08) was 45% lower ($p<0.05$) than the activity in cytosol prepared from adjacent unobstructed liver (mean ± SE, 0.55 ± 0.08) (Table 1). Densitometric analysis of polypeptide bands corresponding to GST proteins (24-28 kD range), separated from cytosol by SDS-PAGE, revealed that the major cytosolic GST subunits were similarly expressed in obstructed and adjacent unobstructed liver.

The influence of various endogenous nonsubstrate inhibitors including chenodeoxycholic acid, deoxycholic acid, bilirubin, biliverdin and hematin, on the activity of cytosolic and affinity purified GSTs towards CDNB and BaP-4,5-oxide was determined *in vitro*. The results are presented in (Table 2). GST activity was inhibited by µmolar concentrations of these inhibitors in a dose-related fashion. Hematin was the most profound inhibitor of hepatic GSTs with an I_{50} value of less than 1 µM. Preincubation of hepatic cytosol with µmolar concentrations of bilirubin resulted in dose-related reductions in GST activity towards BaP-4,5-oxide, however, CDCA and hematin had no effect.

Binding of BaP to DNA and protein *in vivo* and *in vitro*. The degree of protection provided by cytosol from liver of various benthic and pelagic fish species against binding of (±)-r-7,t-8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (anti), (BPDE), to DNA was examined *in vitro*. Liver cytosol from pelagic fish species provided less protection against binding of BPDE to DNA compared to cytosol from the liver of benthic species (Fig. 3). The effect of cholestasis on GST-mediated detoxification of electrophilic BaP metabolites was assessed in various binding assays *in vivo* and *in vitro* (Table1). Hepatic affinity purified and cytosolic GSTs from white suckers provided dose-related protection against the binding of the ultimate carcinogenic metabolite BPDE to DNA *in vitro*. The reduced DNA binding provided by affinity-purified cytosolic GSTs in the presence of GSH was not evident in the absence of GSH. The

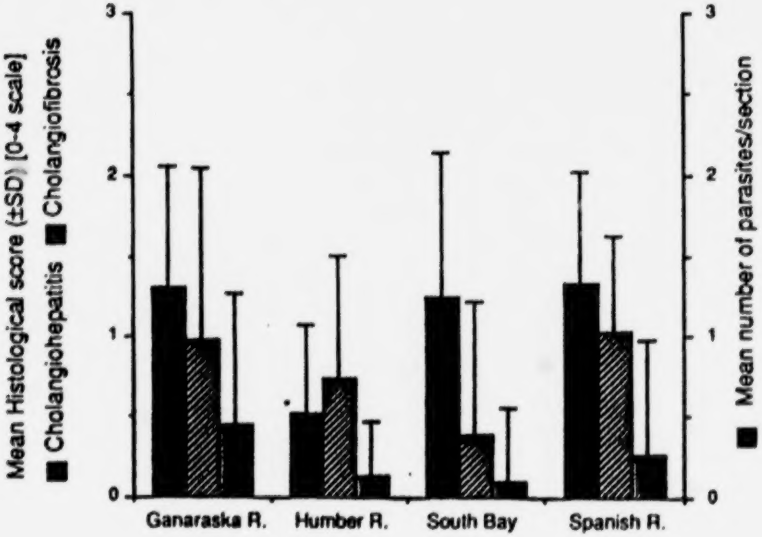
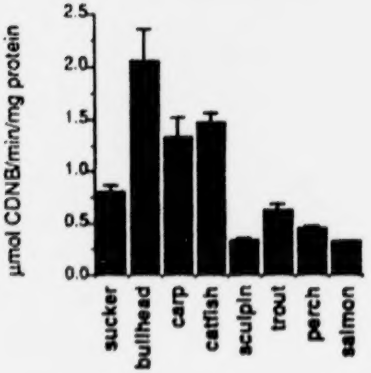
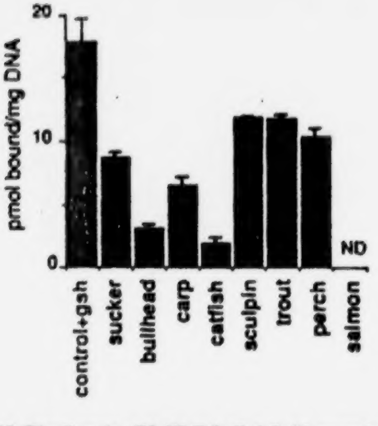


Fig. 1. Severity of lesions and numbers of parasites in inflammatory liver disease in white suckers from various sites in the Great Lakes. Histological sections (5μm) of liver from white suckers from Lake Ontario (Humber R., N=46 and Ganaraska R., n=40) and Lake Huron (South Bay, Lake Huron, N=47 and Spanish R., N=46) in the Great Lakes were stained with H&E and were examined for the presence of inflammatory lesions of cholangiohepatitis or cholangiofibrosis. Lesions were graded on a scale of 0 to 4 (0: no visible lesions; 1: mild; 2: moderate; 3: marked; 4: severe) and the number of parasites visible per section was also noted. Because parasites were rarely encountered, the numbers collectively represent flukes *(Diginea: Sanguinicola)*, nematodes *(Spirurida: Acuarioidea)*, and protozoa *(Myxozoa: Myxosporea)*., all of which were present in some fish from all sites.

Fig. 2

Hepatic Cytosolic Glutathione S-transferase Activity in Various Fish Species





DNA Binding by B[a]P-7,8-diol-9,10-epoxide in the Presence of GSH and Dialysed Liver Cytosol from Various Fish Species

protective effect of cytosol, derived from obstructed or adjacent unobstructed liver, against the binding of BPDE to DNA *in vitro* was also analyzed (Table 1). Cytosol from adjacent liver had slightly stronger protective activity against DNA binding by BPDE (16.4 pmol BPDE/mg DNA ±1.3, $p<0.06$) than cytosol from obstructed liver (20.6 pmol BPDE/mg DNA ±1.6). There was no statistically significant difference in the degree of binding of BaP metabolites to macromolecules (either protein or DNA) in obstructed liver and adjacent unobstructed liver (Table 1).

DISCUSSION

The results of this study clearly indicate that the activity of cytosolic GSTs varies considerably in liver from various fish species from the Great Lakes. The greater GST activity in livers of bottom-dwelling fish might be interpreted as an evolutionary adaptation to provide resistance to the potentially genotoxic and carcinogenic effects of xenobiotic compounds, that are necessarily detoxified by GSTs, to which these fish are exposed in their benthic environment. This interpretation is supported by the finding that liver cytosol from benthic fish species provided greater protection against DNA binding by the carcinogenic PAH metabolite BPDE. This variation in constitutive GST-mediated protection in fish might explain differences in susceptibility of various fish species to PAHs in contaminated sediments. Recent evidence from epidemiological and laboratory studies in various bottom-dwelling fish support the hypothesis that reduced GST-mediated protection might explain differences in susceptibility to some environmental carcinogens. For example, English sole *(Parophrys vetulus)* show a higher prevalence of liver cancer compared to starry flounder *(Platichthys stellatus)* from industrially polluted estuaries of Puget Sound, Washington (17, 18). Studies of BaP metabolism in these fish have demonstrated that *in vivo* binding of ^{3}H-BaP to hepatic DNA is approximately twice the level in English sole compared to Starry flounder (19). HPLC analysis has revealed a much greater proportion of biliary BPDE-GSH conjugates in the bile of Starry flounder than in English sole (18) .Also, hepatic GST (CDNB) activity is three-fold higher in Starry flounder compared to English sole (19). Similarly, tumor surveys of Great Lakes fish indicate a higher frequency of liver tumors occurs in bottom-dwelling brown bullheads *(Ictalurus nebulosus)* compared to carp *(Cyprinus carpio)* collected from the same polluted areas in Lake Erie (20, 21). Recent comparative studies of BaP-7,8-diol metabolism in hepatocytes isolated from brown bullheads and carp revealed that carp hepatocytes metabolized BP-7,8-diol faster than bullhead hepatocytes producing a larger proportion of GSH conjugates (22). However, higher levels of BaP-DNA adducts which occur in carp hepatocytes fail to explain higher susceptibility of brown bullhead to PAH-associated liver cancer.

In some white suckers, from various sites in the Great Lakes, a parasitic obstructive hepatobiliary disease is associated with severe regional cholestasis of distal liver lobes. The nature of this segmentally obstructive liver disease provides an opportunity in which to evaluate acquired alterations in GST activity and regional differences in carcinogen metabolism in a unique natural model of inflammatory liver disease. The results of these experiments indicate that obstructive liver disease and cholestasis in white suckers reduces hepatic glutathione S-transferase activity for CDNB as a substrate. Because amounts of GST subunits were similar, this reduction appears to be due to an inhibitory effect of some substance in cholestatic liver. During cholestasis, the intracellular concentration of organic ions, such as bile acids and bile pigments, increase to levels which can impair the catalytic activity of GSTs (23, 24, 25). Heme catabolites, namely hematin, biliverdin, and bilirubin, and also bile acids namely CDCA and DCA, inhibited CDNB conjugating activity of white sucker cytosol and purified GSTs *in vitro.* This suggests that some bile acids and bile pigments that accumulate during cholestasis may contribute to the lower activity measured.

An important issue that is addressed in this study is whether inhibition of GSTs , that is associated with bile duct obstruction and cholestasis in white suckers, alters the ability of GST isoenzymes to detoxify carcinogens such as BaP. The present experiments indicate that bilirubin is one endogenous nonsubstrate ligand capable of inhibiting cytosolic GST activity towards BaP-4,5,oxide *in vitro.* This interpretation is supported by moderate but significantly less protection against the binding of BPDE to DNA in cytosol from obstructed liver compared with adjacent unobstructed liver. However, the levels of binding of BaP metabolites to macromolecules in fish given ^{3}H-BaP *in vivo* indicate that cholestasis had neglible influences on binding of ^{3}H-BaP to

Table 1. *Inhibition of white sucker hepatic GSTs by various endogenous nonsubstrate ligands*

	I_{50} (µM)	
Inhibitor	Cytosol	Affinity Purified GST
DCA	240.0	267.0
CDCA	75.0	115.0
Bilirubin	N.D.	18.8
Biliverdin	12.5	6.3
Hematin	0.6	0.4

The I_{50} value is the concentration of inhibitor giving 50% inhibition of the enzyme activity at pH 7.5 with 0.5 mM CDNB as a substrate in the presence of 2 mM GSH. The inhibition studies were performed by preincubating the enzyme with inhibitor for 5 minutes at 25°C prior to the addition of substrates. Experimental details are outlined under Methods. The specific activities (µmol CDNB/min/mg protein) of the GST enzymes were: cytosolic GST, 0.75 ±0.06; affinity purified GST, 6.9 ±1.1. Values represent the means of three determinations which were within 5%. N.D. (not determined), bilirubin failed to reduce the cytosolic GST activity by 50% at the highest concentration assayed (100 µM).

Table2. *Differences in cytosolic GST activity, cytosolic GST-mediated protection against BPDE-DNA binding in vitro and BaP-macromolecular binding in vivo in obstructed and surrounding liver in White suckers*

	Liver Region			
	N	Obstructed	N	Surrounding
GST activity (CDNB)[a]	6	0.25 ±0.08[e]	6	0.55±0.08
BaP-macromolecular binding				
In vitro				
BPDE-DNA	6	20.6 ±1.6	6	16.4 ±1.3
In vivo[d]				
BaP-protein	4	7.6 ±3.0[f]	4	4.6 ±1.9
BaP-DNA	4	0.24 ±0.1	4	0.25 ±0.2

[a] Values expressed as µmoles CDNB/min/mg protein represent the mean ±SE of three determinations.

[c] Values, expressed as pmoles ^{3}H-BPDE bound/mg DNA/8 min. ^{3}H-BPDE was incubated in the presence of 0.2 mg calf thymus DNA, NADPH (2mM), GSH (2.5 mM) and 1.6 mg cytosolic protein (from obstructed or surrounding liver). Values represent the mean ±SE of two determinations.

[d] Values are expressed as fmoles BaP bound/mg protein or DNA and represent the mean ± SE of 4 determinations. Suckers, with obstructions visible on laparotomy, were orally administered 5 µCi ^{3}H-BaP (56 Ci/mmol). Livers were sampled at 24h, DNA and protein were isolated and quantified and BaP-derived radioactivity was measured by LSC.

[e] Statistically different from cytosol from surrounding liver ($p \leq 0.05$)

[f] Statistically different from protein adducts in surrounding liver ($p \leq 0.06$)

hepatic DNA. This suggests that the significance of GST inhibition on carcinogen detoxification *in vivo* may not easily be resolved.

The presence of parasites and parasitic lesions in fish with segmentally obstructive liver suggests that parasitism may be directly involved in the pathogenesis of obstructive bile duct disease. The chronic inflammatory bile duct disease is widespread in white suckers in the Great Lakes and not consistently associated with hepatic neoplasms which are found preferentially in industrially polluted locations. This suggests that parasitic liver disease is not exclusively responsible for neoplastic development, but it cannot be dissociated from the neoplastic disease because the parasitic disease is endemic in all sites. Thus the disease might be an important influencing factor in the pathogenesis of liver neoplasia in these fish. Concurrent nonneoplastic liver lesions, including inflammatory, degenerative and necrotic lesions, and neoplastic hepatic lesions have also been observed in other studies of pollution-associated neoplasia in both marine and freshwater bottom-dwelling fish (26, 27, 28, 29). However, parasitism was apparently not associated with nonneoplastic lesions observed in English sole from Puget Sound (26) nor in winter flounder from Boston Harbour (28). While it is not possible to determine, from the present study alone, the role of parasitic disease in the development of liver neoplasms in white suckers from polluted environments, there is substantial epidemiological evidence supporting a correlation between HCC and inflammatory liver disease in humans infected by Hepatitis B virus or various parasites (31). However, the cellular and molecular mechanisms underlying this association are unclear (32). Increased proliferation of cholangiolar and bile duct epithelium in fish with cholangiohepatitis and cholangiofibrosis might be an important contributor to the co-occurrence of liver neoplasia and bile duct disease in white suckers. However, chronic inflammation and liver cell injury might increase susceptibility to liver carcinogens by altering activation, detoxification or biliary excretion of carcinogens, or altering DNA repair mechanisms (33, 34). The reduction in GST activity, in association with inflammatory bile duct disease and cholestasis, might represent another mechanistic explanation that does not exclude the possiblilty that the increased rate of proliferation and other influences may also be involved. The results of this study do suggest that while bottom-feeding white suckers are remarkably resistant to BaP, parasite-associated bile duct disease could interfere with detoxification of other xenobiotics detoxified by GSTs. Further studies are required to determine which environmental carcinogens are most likely more harmful to altered livers of white suckers.

Because of the complexity and multifactorial nature of pollution-associated hepatocarcinogenesis in bottom-dwelling fish, the relevance of this problem with respect to assessing the status of the aquatic environment is not easily established. As a result, the use of tumor surveys in wild fish, without supportive data from mechanistic studies, might lead to the erroneous interpretation of epidemiological studies or implementation of ineffective or irrelevant remedial action. By investigating factors which influence the development of liver cancer in various fish and mammalian models of hepatocarcinogenesis the extrapolation of risk of cancer development in humans might be made with greater confidence. For example, variations in constitutive resistance to carcinogens between various species of fish, and between individuals in human populations, suggests that the risk of developing cancer is not uniform in heterogenous populations. Questions such as "are humans similarly sensitive to environmental carcinogen X, as rats, or mice, or white suckers or some other species?" are important in the context of how risk assessment is currently performed. However, recognition of constitutive differences within and among species in the expression and function of resistance systems, or aquired differences associated with concurrent disease or exposure to competitive inhibitory or inducing substances, presents a complex situation that cannot be handled optimally with simple regulatory rules based on simple assumptions. Furthermore, interactions between various environmental, endogenous, or dietary substances in the pathways involved in biotransformation and detoxification might increase the sensitivity of individuals to carcinogens to which they are normally resistant. The ultimate challenge is to increase our understanding of the complex carcinogenic process and to more reliably predict and estimate cancer risk in human populations. Recognitions of the conceptual and practical obstacles in the way of this objective, and the limitations in approaches based on simpler notions of carcinogens as health hazards, is an important stage in this pursuit.

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DOES EXPOSURE TO GENOTOXINS INFLUENCE GENETIC DIVERSITY IN NATURAL FISH POPULATIONS?

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Although the acute toxicities of contaminants on Great Lakes biota has been widely researched (Sergy 1987), the effects of chronic exposure to contaminants are much less understood. Biological effects resulting from toxic chemical exposure include reproductive impairment and tumour induction in fish populations, as well as the induction of heritable mutations must be determined.

This study evaluated the applicability of mitochondrial DNA (mtDNA) diversity as a model for assessing the genetic impact of contaminant exposure on natural populations. A common Great Lakes benthivore, the brown bullhead (Ictalurus nebulosus), was chosen as the study species as it often lives in contact with high sediment levels of mutagenic polycyclic aromatic hydrocarbons (PAHs). A strong relationship has already been demonstrated between sediment PAH levels and chemically-induced somatic tumour frequencies in feral populations of this species (Black 1988). Studies on the brown bullhead further indicate its high capacity to bioaccumulate and activate PAHs (Baumann and Harshbarger 1985, Steward et al. 1990). The highest tissue concentrations are found in the gonads (Steward et al. 1990). Evidence suggests that PAHs preferentially bind to mtDNA (Backer and Weinstein 1980) to induce mutations via base substitutions (Eisenstadt et al. 1982). These results jointly suggest that brown bullheads are likely to be subjected to chemically-induced mutations to mtDNA in the germ line that will be passed on to subsequent generations.

A positive relationship between mtDNA diversity and contaminant levels would suggest that mutation rates are elevated at highy impacted sites. A negative relationship would imply that exposure to contaminants selectively reduces genetic diversity or that impacted populations are subject to more bottlenecking events than relatively unimpacted populations. A lack of association would indicate that contaminant exposure does not influence detectable mtDNA variation.

Nine populations of brown bullhead were sampled across the lower Great Lakes (Fig. 1) with fish obtained from five highly contaminated sites (Gibraltar Bay, Black River, Cuyahoga River, Ashtabula River, and Hamilton Harbour) and from four relatively pristine sites (Point Pelee, Huron River, Old Woman Creek, and Cape Vincent). Using sixteen restriction enzymes, over sixty unique mtDNA haplotypes were identified among 240 fish. To reduce the effects of variation in sample size, the haplotype diversity in each population was estimated using Simpson's diversity index (Table 1). The diversity estimate for each highly contaminated site was compared to the nearest neighbouring pristine site of similar habitat type (river or bay).

The results of the analysis establish that fish from contaminated sites show significantly lower levels of mtDNA diversity than fish from clean sites ($p \leq .05$). This result suggests that contaminant exposure reduces mitochondrial genetic diversity of feral fish populations. We suggest that this reduction in diversity is a consequence of genetic bottlenecking associated with population collapses triggered by contaminant exposure. Further study is required to examine mtDNA diversity within the bodies of single fish to more firmly assess the mutagenic impacts of contaminant exposure.

Figure 1. Sampling sites of brown bullhead populations. Relatively uncontaminated sites○: Point Pelee (PP), Huron River (HR), Old Woman Creek (OWC), Cape Vincent (CV). Highy contaminate sites●: Gibraltar Bay (GB), Black River (BR), Cuyahoga River (CR), Ashtabula River (AR), Hamilton Harbour (HH).

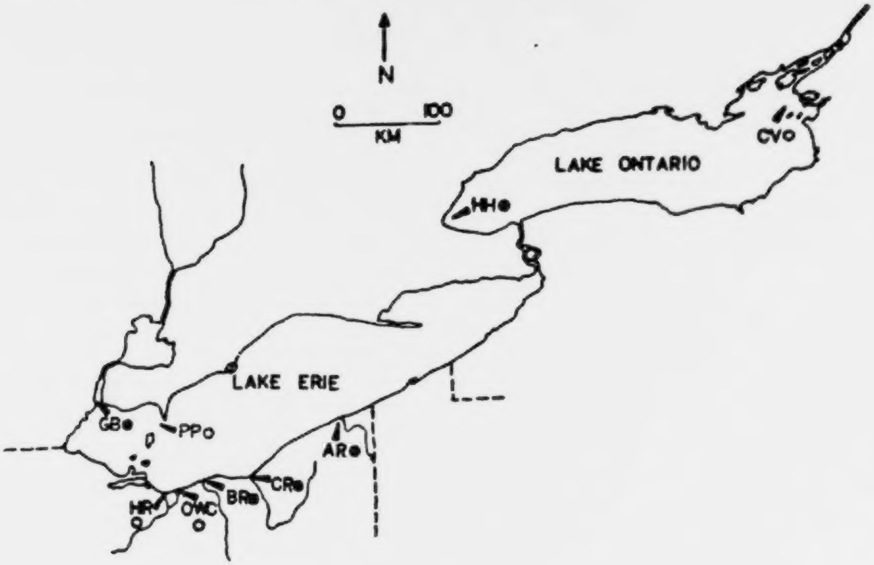

Table I. Mitochondrial DNA haplotype diversity in brown bullhead populations at highly contaminated (H) sites and relatively uncontaminated (L) sites.

Population	n	Haplotype diversity	Contaminant status
PP	22	5.35	L
HR	27	3.74	L
OWC	15	4.78	L
CV	30	8.62	L
GB	27	2.44	H
BR	29	1.35	H
CR	25	1.37	H
AR	28	3.37	H
HH	29	5.56	H

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CARACTÉRISATION DE LA RÉPONSE D'UN INDICATEUR CYTOCHIMIQUE DE POLLUTION FACE AUX FACTEURS ENVIRONNEMENT NAUTRELS.

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L'examen d'aspects spécifiques des fonctions cellulaires offre un moyen rapide et sensible d'indiquer qu'elles sont les réponses aux stress environnementaux (Moore, 1980). La mesure de la stabilité de la membrane lysosomale est reconnue comme un excellent indice cellulaire reflétant l'état de stress en général subi par un organisme (Bayne, 1986). En plus, cet indice permet de quantifier le niveau de stress (Lack et Widdows, 1986). Ainsi une diminution de la membrane lysosomale chez les bivalves, spécialement chez *Mytilus edulis*, a été mise en évidence en réponse à de nombreux stress expérimentaux tant physiques que chimiques. Ces facteurs provoquant un stress sont aussi variés que l'hyperthermie, l'hypoxie, l'hyposalinité, l'induction de la ponte, l'injection d'hydrocarbures polycycliques aromatiques et d'ions métalliques (Bayne, 1986). Cet indicateur de stress a aussi été utilisé lors d'études écotoxicologiques *in situ* afin de déterminer le niveau de stress induit par des contaminants présents dans le milieu (Pelletier et Pellerin, 1990). Cependant aucune étude indiquant le niveau de stress déterminé à l'aide de cet indice, pouvant être induit par les facteurs environnementaux naturels n'a encore été effectué. Les invertébrés sessiles habitant la zone intertidale sont sujets à de considérables fluctuations environnementales naturelles associées principalement avec le cycle des marées. Ainsi, ces organismes sont sujets à de grandes fluctuations de température, un temps de nutrition réduit, l'effet des vagues et l'exposition à l'air qui peut entraîner la dessiccation (Widdows et Shick, 1985, Blackstock, 1984). Nos objectifs sont donc de vérifier la variation de la déstabilisation de la membrane lysosomale dans un milieu non soumis à des stress de nature anthropique et ainsi caractériser la réponse de la membrane lysosomale face aux paramètres physiques du milieu intertidal.

Après 15 jours d'acclimatation des moules (*Mytilus edulis*) et des myes (*Mya arenaria*) transplantées dans l'Anse à l'Orignal, Parc du Bic, Québec, l'échantillonnage s'est déroulé durant deux jours avec une fréquence de prélèvement aux deux heures. Pour les deux espèces le cycle des marées semble avoir un effet dramatique sur la stabilité de la membrane lysosomale (figure 1). Ainsi lorsque les individus sont en période d'émersion la membrane lysosomale est déstabilisée plus rapidement, indiquant un stress plus important, qu'en période d'immersion (myes: test de Student= 3.491, DL= 22, $p < 0.01$; moules: test de Student= 2.571, DL= 21, $p < 0.05$). De plus les corrélations entre la période de labilisation, indiquant le niveau de stress, et la hauteur des marées sont significatives (moules: r=0.585, test de Student= 3.302, p< 0,01; myes: r= 0.471, test de Student= 2.502, p< 0.05).

L'exposition à l'air induit une déstabilisation rapide de la membrane lysosomale, nous indiquant un stress important. Ainsi un facteur environnemental naturel, tel l'émersion, donne la même réponse de cette indice de stress que l'injection d'une substance toxique comme le phénanthrène (Moore et Viarengo, 1987). Chez *Mya arenaria* et *Mytilus edulis* la réponse de la membrane lysosomale face à l'émersion est identique, même si l'adaptation développée par les deux organismes pour survivre en période d'exposition à l'air est différente. Leur adaptation comportementale et métabolique dépend principalement de leur habitat (Widdows et Shick, 1985). Ainsi la moule étant épibenthique, elle se referme pour éviter la dessiccation, l'amenant à utiliser un métabolisme anaérobique. Quant à la mye, l'habitat endobenthique minimise la dessiccation et permet aux organismes de maintenir un métabolisme aérobique en utilisant l'oxygène de l'air (Blackstock, 1984). Ce métabolisme ne semble donc pas avoir d'effet direct sur la déstabilisation de la membrane lysosomale. De plus même si les organismes sont adaptés aux période d'exposition à l'air, il semble qu'ils subissent toutefois un stress, qui pourrait expliquer les variations de croissance observées selon le niveau altimétrique.

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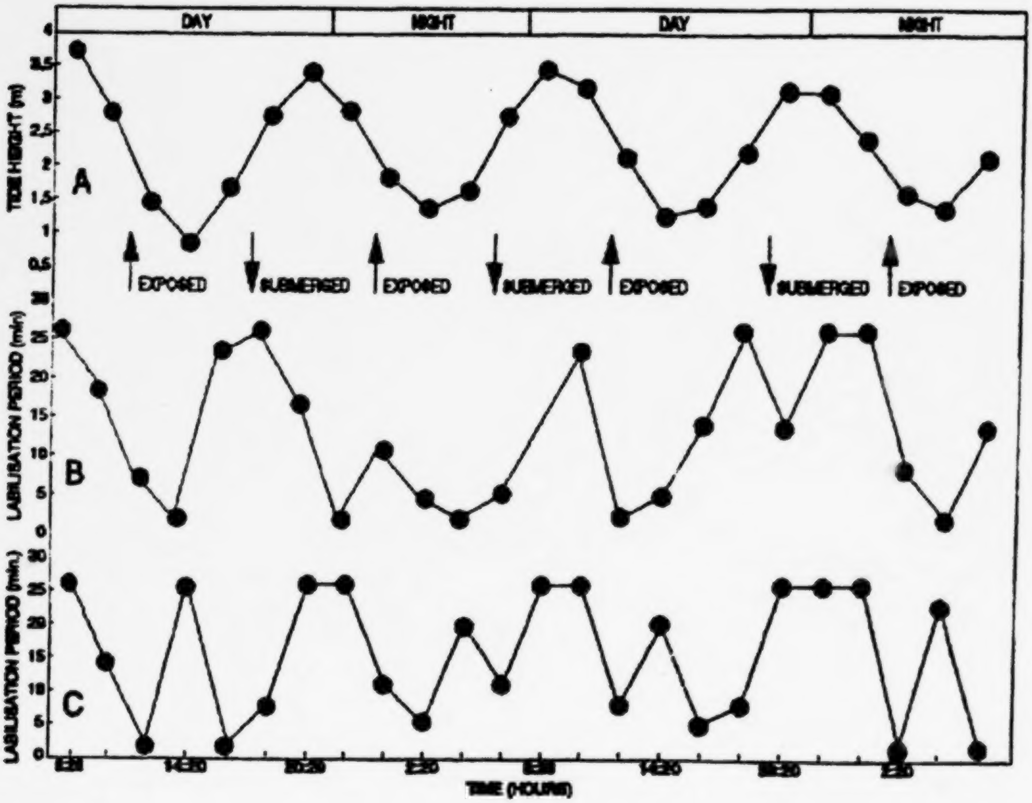

Figure 1: Changement de la période de labilisation chez *Mytilus edulis* (B) et *Mya arenaria* (C) en relation avec la hauteur de la marée (A).

AN INTERLABORATORY COMPARISON OF THE ETHOXYRESORUFIN-O-DEETHYLASE ASSAY FOR MFO ACTIVITY.

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Abstract

Samples of minced liver were collected from wild white sucker (Catostomus commersoni) exposed to effluent from a bleached kraft pulp mill (BKME) and from a reference site. The samples were randomly numbered and were delivered to 14 laboratories, in Canada and the United States. Samples received by 2 laboratories (4,6) were not valid due to shipping problems. All laboratories performed analysis on microsomal preparations for ethoxyresorufin-o-deethylase (EROD) activity without any a priori attempts at standardization of techniques or assay conditions. Analyses indicated that a) all labs successfully differentiated all high activity (BKME) and low activity (reference) samples; b) the induction (exposed divided by reference fish) varied from 10.4-fold to 20.7 fold, although activity detected from the induced site varied from 20 to 298 p mol min^{-1} mg^{-1}; c) the induction detected was not affected by whether the labs used post-mitochondrial supernatants (10,000xg) or microsomes (100,000xg); and d) induction detected was not affected by storage time, pH, buffer, assay time, or assay temperature, even though these conditions affected the absolute activity of the samples.

Introduction

Hepatic mixed-function oxygenase (MFO) enzyme activity has been proposed as an indicator for delineating zones of exposure to inducing agents [1,2]. Induced MFO activities have been found in fish downstream of bleached kraft mills in Ontario [3-5], other Canadian provinces [6,7] and Scandinavia [8-10]. Over the past three years, we have been studying the impacts of bleached kraft mill effluent (BKME) on Jackfish Bay, Lake Superior; Jackfish Bay received untreated or primary-treated BKME from 1949 until 1989. The study site is isolated and receives no other industrial or domestic effluents.

Over the past three years, there has been a consistent and marked difference in mixed function oxidase activity between sites (measured via EROD, as well as AHH reduction of diphenyloxazole (PPO) and benzo(a)pyrene (B(a)P)) [11]. This study collected samples from Jackfish Bay (48°50'N, 86°58'W) and a reference site (Mountain Bay, 48°56'N 87°50'W), and liver samples were analyzed by 11 different laboratories for MFO activity as determined by the ethoxyresorufin-o-deethylase (EROD) assay.

Methods

Fish were collected by gillnet, and livers were removed from live white sucker (Catostomus commersoni) after removal of the intact gall bladder. Samples from two fish were minced simultaneously, mixed, rinsed with 0.15 M KCl, divided into 14 equal aliquots and frozen in liquid nitrogen. Each individual sample consisted of minced liver from two fish; sample pooling was necessary because of the variability in MFO activity within livers, and due to the small size of the livers at the reference site.

The samples were sorted on dry ice and stored at -80°C until shipment to the laboratories on dry ice. Samples included six tubes from a reference site and six from Jackfish Bay, and numbers were assigned randomly. Samples were delivered to 14 laboratories, which are identified only by number. Of these 14 laboratories, samples received by 2 laboratories (4,6) were not valid. At both laboratories samples were not maintained at sufficient temperatures for the assay, due to a) delays in delivery by courier (samples were shipped via ground although instructions were for air transportation) and b) delays during processing for customs clearance.

EROD assay conditions at each laboratory are described in Table 1. There were no attempts to a priori standardize incubation conditions for the assay, which resulted in some laboratories processing the samples as post-mitochondrial 10,000 x g supernatants (PMS) and some assays using 100,000 x g microsomal preparations; two laboratories analyzed samples using both preparations. Assay conditions, including temperature and pH varied dramatically between techniques (Table 1). Samples were shipped to laboratories in late August, 1990, and analyses by individual laboratories were conducted between August, 1990 and March, 1991.

Results and Discussion

All laboratories successfully differentiated all high activity (BKME-exposed) and low activity (reference) samples. Due to a sorting mix-up, lab 5 received duplicates of a high activity sample and only 5 low samples, while lab 13 received duplicates of a low activity sample and only 5 high activity samples. Although absolute activity at the high activity site ranged from 20 p mol (min^{-1} mg $protein^{-1}$ resorufin) to 298 p mol, the relative induction (exposed divided by reference fish) was remarkably constant. Induction ranged only over a factor of 2 (from 10.4-fold to 20.7 fold). Furthermore, 8 of the 10 labs were within the range of 13.2 to 18.5-fold.

The induction detected was not affected by whether the labs used PMS (Table 2) or microsomes (Table 3) (7 labs with PMS yielded 15.5-fold while 5 labs doing microsomes detected 17.0-fold induction). Two laboratories (2 and 3) performed EROD using both PMS and microsomes, and found no detectable difference in the relative induction between the two methodologies. However, microsomal preparations yielded 3.5 times higher activity, in both BKME and reference samples.

The induction detected was not affected by whether samples were analyzed immediately or after prolonged storage. Two laboratories analyzing samples in February found induction of 18.2 and 15.8, while the average found by laboratories analyzing in August or September ranged from 13.2 to 17.0.

Additional experiments on these white sucker samples (van den Heuvel and Munkittrick, unpubl. data) found that optimal pH for white sucker microsomes was 7.8, with the activity declining rapidly above 8.0 or below 7.5. The assay was stable for white sucker microsomes within a range of 20 to 30°C, with activity declining very rapidly above 30. Further effects on activity were found by omitting BSA (higher activity) or Mg (lower activity) from the reaction.

In spite of the observations that some laboratories utilized assay conditions clearly outside the optimal ranges for white sucker liver samples, there was no correlation between non-optimal conditions and the degree of relative induction detected. The EROD assay appears to be extremely robust, with low and high activity samples being equally affected by adverse incubation and handling conditions.

Acknowledgements

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Table 1. Interlaboratory EROD methodology comparison (labs 4 and 6 lost their samples prior to analysis).

Lab	Buffer	pH	Mg^{++} mM	BSA mg mL^{-1}	NADPH mM	7-ER µM	Carrier	Protein mg/mL	Protein Method	Volume	Reaction Mixture (1,2,3,4,5,6)[1]	Time min	Temp °C
1	0.1 M HEPES	7.8	9	1.4	1.4	1.3	DMSO	0.8	Lowry	1.41	1250,10,50,30,20,50	4	25
2	0.1 M HEPES	7.8	17	1.4	0.5	1.7	DMSO	0.8	BioRad	1.43	1250,20,50,30,30,50	10	25
3	0.1 M HEPES	7.8	10	1.7	1.0[2]	1.6	DMSO		Lowry	1.25	1110,10,50,30,10,50	2	25
5	0.1 M KH_2PO_4	8.0	-	-	0.12	0.26	PO_4 buffer	0.4	Biorad	2.0	1690,0,0,10,100,200	10	30
7	0.1 M TRIS + 0.1 M NaCl	8.0	6.3	1.0	0.77[2]	2.0	MeOH	0.1	Lowry	1.0	640,0,100,50,10,200	15	37
8	0.1 M TRIS	8.5	-	-	0.008	1.0	TRIS/DMSO		BioRad	2.0	0,0,0,20,1970,10	2	37
9													
10	0.1 M HEPES	7.8	10.9	1.8	0.5	1.5	DMSO	0.8	BioRad	1.14	1000,10,50,20,10,50	2	25
11	0.1 M TRIS + 0.1 M NaCl	8.0	-	-	0.5	2.0	???	3 - 8					25
12	0.1 M HEPES	7.8	9	1.4	0.7	0.9	DMSO		Biuret	1.4	1250,10,50,30,10,50	2	25
13	0.05 TRIS	8.0	90	-	0.56	0.25	TRIS		Lowry	2.0	1820,0,0,30,100,50	10	25
14	0.05 TRIS/ sucrose	7.5	-	-	0.15	1.8	???	0.05	Lowry	1.25	1060,0,0,125,15,50	15	27

[1] Buffer, Mg, BSA, NADPH, ER, Protein in µL

[2] NADPH generating system, calculated as maximum NADPH

Table 2. Data for samples analyzed as Post-mitochondrial supernatants.

Sample		Lab						
Number		1	2	3	5	10	12	13
Mountain								
	6	3.34	4.51	3	1.029	5.48	0	2.9
	8	2.71	5.73	4		9	0.1	2
	9	6.77	10.11	8	4.852	16.75	7.5	3.1
	11	4.34	7.82	5	1.263	8.89	7.22	3.2
	14	3.5	7.95	7	1.587	14.87	8.44	3.1
	17	3.66	3.94	4	1.736	7.51	1.42	1.95
average		4.053	6.677	4.833	2.093	10.41	4.113	2.708
Jackfish								
	5	38.7	135.5	92	39.1	194.9	130.5	46.8
	7	57.7	91.2	111	32.51	202.2	92.9	
	10	38.2	54.5	51	20.93	119	102.7	17.3
	12	100.4	97.2	88	45.88	184.2	138.2	44.9
	16	79.3	66.8	82	38.88	115.3	148.8	47
	18	102.1	99.4	93	36.06	170.8	111.6	45.2
average		69.07	90.77	86.17	35.56	164.4	120.8	40.24
Induction		17.04	13.59	17.83	16.99	15.78	29.36	14.86

Table 3. Data summary for samples analyzed as microsomal preparations.

Sample		Lab					
Number		2	3	7	8	11	14
Mountain							
	6	7.3	9	1.18	4.09	27	7.9
	8	15.4	15	2.34	4.61	18	9.15
	9	34	25	6.17	8.73	25	21.55
	11	25.3	21	7.7	4.06	13	10.65
	14	33.4	22	4.86	7.03	0	10.8
	17		14	3.45	4.34	12	8.25
average		23.08	17.67	4.62	5.477	15.83	11.38
Jackfish							
	5	367.7	330	73.67	144.7	620	314.5
	7	213	422	67.76	152.5	460	222.1
	10	154.3	139	42.68	56.51	170	119
	12	363.3	331	144.3	105.9	410	199.3
	16	274.8	222	75.23	60.27	300	118.1
	18	445.8	341	100.2	161.3	470	292.6
average		303.2	297.5	83.97	113.5	405	210.9
Induction		13.13	16.83	18.18	20.72	25.58	18.53

HISTOPATHOLOGIE DES BRANCHIES ET DU FOIE DE POISSON COMME INDICATEUR DE LA QUALITÉ DES HABITATS AQUATIQUES DU SAINT-LAURENT.

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INTRODUCTION

Les altérations histopathologiques ont été étudiées principalement chez les mammifères (Meyers et Hendricks 1985). Leur utilisation comme indicateur de stress environnemental chez les poissons est plus récente et en plein développement (Hinton et Laurén 1990). Ainsi, des mesures histologiques quantitatives sur les branchies peuvent servir d'indicateur de la qualité de l'environnement puisqu'elles sont continuellement exposées aux polluants (Mallat 1985). De même, l'observation des tissus hépatiques peut être utile pour observer les effets toxicologiques de certains contaminants (Hinton et Laurén 1990). Ce projet, s'incrivant dans le Plan d'action Saint-Laurent, a comme objectif principal d'évaluer l'utilité de l'histopathologie des branchies et du foie comme indicateur de l'état de l'environnement et d'examiner la pertinence d'appliquer l'histopathologie dans un éventuel suivi de la qualité des ecosystèmes du Saint-Laurent.

MATÉRIEL ET MÉTHODES

Des poissons de trois lacs riverins (Saint-François, Saint-Louis, et Saint-Pierre), de l'estuaire moyen (entre Québec et la rivière Saguenay) et de trois lacs témoins (Berthe, Massawippi et Grandin) ont été échantillonnés en septembre 1989 et 1990. Les espèces étudiées sont le Meunier noir (*Catastomus commersoni*), le Grand Brochet (*Esox lucius*), la Barbotte brune (*Ictalurus nebulosus*) et la perchaude (*Perca flavescens*) dans les lacs et l'Éperlan arc-en-ciel (*Osmerus mordax*) et le Poulamon atlantique (*Microgadus tomcod*) dans l'estuaire. Les tissus de branchie et de foie ont été fixés avec du cacodylate-glutaraldéhyde et enrobé dans de l'épon et des coupes histologiques de 0.5 μm ont été effectuées. Les paramètres suivants ont été quantifiés sur plusieurs coupes par poisson:

Branchies:

- distance inter-lamellaire;
- épaisseur des lamelles;
- épaisseur de l'épithélium lamellaire;
- distance de diffusion;

- nombre de cellules à mucus par unité de surface de filaments;
- nombre de cellules à chlorure par unité de surface de filaments;
- nombre de cellules à mucus par unité de surface de lamelles;
- nombre de cellules à chlorure par unité de surface de lamelles;

Foie:

- nombre de centres de mélanomacrophages par mm^2;
- rapport surface centres de mélanomacrophage/surface tissu hépatiques.

RÉSULTATS

La grande majorité des tissus observés possède des altérations indicatrices de stress environnemental et ceci, pour tout le Saint-Laurent. Toutefois, il est difficile de juger de l'ampleur du problème puisque les poissons des sites témoins sont aussi perturbés. Au niveau spécifique, le Meunier noir, dans la partie ouest du lac Saint-François, présente des tissus hépatiques et branchiaux très affectés. Pour le Grand brochet, les tissus hépatiques sont également affectés à l'ouest du lac Saint-François, tandis que certains paramètres des branchies (distance interlamellaire et cellules à mucus) sont touchés dans le lac Saint-Pierre. Le Poulamon atlantique, au niveau de l'estuaire, présente des nombres plus élevés de cellules à chlorure et des distances interlamellaires plus petites sur la rive sud que sur la rive nord. Une analyse en composante principale a permis de séparer les espèces de poissons selon l'intensité des altérations, le Meunier noir étant plus affecté. D'autres analyses en composante principale par espèce ont montré que les poissons les plus altérés ne provenaient pas des sites témoins mais bien de sites du Saint-Laurent connus pour leur piètre qualité environnementale.

DISCUSSION

Les mesures histopathologiques sur les branchies et le foie de poissons du Saint-Laurent indiquent qu'il y a des stress environmentaux à tous les sites mais quelques sites (e.g. Beauharnois, Cornwall) sont plus particulièrement affectés. Toutefois, il est difficile de juger de l'ampleur des altérations les mesures sont relativement variables à chaque site, ce qui peut nuire à leur différenciation sur une grande échelle spatiale comme le Saint-Laurent. Pour compenser cet inconvénient, il faudrait augmenter la taille des échantillons afin de réduire la variance et améliorer la puissance des tests statistiques. Cependant, l'effort requis pour l'analyse quantitative de coupes histologiques est énorme. Les résulats d'analyses semi-quantitatives effectuées aux même sites en 1990 ont fait ressortir les mêmes tendances et ceci, avec un effort d'analyse dix fois monidre. L'utilisation de l'histopathologie dans un éventuel suivi de la qualité des habitats du fleuve, est intéressante puisque cet indicateur intègre plusieurs facteurs de stress. Toutefois, il serait préférable de limiter la précision des analyses à une échelle semi-quantitative puisque la qualité résultats n'est pas affectée significativement. Enfin, la sélection de sites témoins adéquats est un élément clé pour bien évaluer l'ampleur des altérations.

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REDUCTION IN PAH CAUSES DECLINE IN LIVER NEOPLASMS IN BLACK RIVER BULLHEAD.

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During the past two decades, numerous studies have documented elevated neoplasm frequencies among populations of benthic fish living in polluted waters. In particular, a cause and effect relationship has been hypothesized for carcinogenic polynuclear aromatic hydrocarbons (PAH) in sediment and liver neoplasms in benthic fishes. This hypothesis has been strengthened by the induction of liver neoplasms in several species exposed to benzo(a)pyrene (B(a)P) in the laboratory. The hypothesis has also been aided by documentation that fish in the wild not only take up B(a)P, but contain carcinogenic metabolites of B(a)P in their bite. We wished to further test the hypothesis by comparing liver tumor frequencies in a population of benthic fish before and after the closure of the principal PAH source.

Adult brown bullhead (Ictalurus nebulosus) from the Black River, Ohio were found to have a high frequency of grossly visible liver neoplasms in 1980, 1981, and 1982. PAH from a USA coke facility were suggested as the cause of these lesions. The steel and coke industry underwent a decline in 1982, and the USA coke plant was closed in October, 1983. Livers were taken from random samples of brown bullhead of age 3 or older captured in 1982 (N=124) and in 1987 (N=80). Concentrations of PAH in whole bullhead were determined during 1981, 1982, and 1983.

Concentrations of PAH such as phenanthrene, fluoranthene, and the carcinogen B(a)P were only one-tenth as high in bullhead sampled in 1982 and 1983 as they had been in bullheads sampled in 1981. Only 20% of the bullhead livers taken in 1982 were normal, while 42% of livers examined in 1987 were normal. The overall liver cancer frequency declined significantly ($p \leq .01$) between 1982 (39%) and 1987 (10%). The decline in liver cancer continued to be significant ($p \leq .01$) between 1982 and 1987 if fish were compared by age group (age 3 and age 4). Similarly, the percentage of normal fish had significantly increased ($p \leq .05$) within each of these age groups. Cellular alteration and non-cancer neoplasms were not significantly different. The latter finding is largely an artifact of the classification system, in which fish were only counted once under the category of their most advanced lesion, even if less advanced lesions were also present.

Both age 3 and age 4 bullhead declined significantly ($p \leq .05$) in hepatocellular carcinoma between 1982 (age 3 = 17%; age 4 = 16%)

and 1987 (age 3 = 2.4%; age 4 = 0%). However, hepatocellular alteration in age 3 fish and non-cancerous hepatic neoplasms in age 4 fish were both significantly higher in 1987 than in 1982. Again, this is most likely an artifact of the classification system. Biliary lesions declined even more dramatically. Biliary cancers were significantly reduced from 19% to 5% for age 3 ($p \leq .05$) and from 33% to 7% for age 4 ($p \leq .01$). Cellular alteration and non-cancerous biliary lesions also declined between 1982 and 1987 for both age groups. These results strongly affirm the cause-effect hypothesis for PAH and liver neoplasms in fish. A reduction in PAH entering the Black River resulted in lowered PAH residue levels in the brown bullhead population. Five years after the decline in residue levels was first noted, liver cancer (both biliary and hepatic) had declined significantly in fish of age 3 and 4. No dredging or other action had been taken prior to the 1987 sample. This indicates that neoplasm frequencies will decline after PAH input ceases, although the complete recovery process may be lengthy.

BIOMARKERS: ACADEMICALLY INTERESTING OR USEFUL INDICATORS. J.H. McCormick, U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota.

For the purpose of this presentation, biomarkers will be considered those phenomena in an individual or population of organisms which indicate that it has undergone a life altering experience which has left an identifiable mark. The relevance of these "marks" must be considered. I propose that their purpose be as "Early Warning" indicators of environmental degradation and that they, in this presentation, be restricted to observations in fish. Biomarkers will be considered some observable and preferably measurable, population, anatomical or physiological parameter. Because environmental degradation can result from may causes, simple or complex, markers to be most useful should not only indicate that a problem exists but also that they identify specifically the cause of that problem. However, most biomarkers are only generic indicators, i.e. they merely indicate that a problem exists; consequently it is necessary to look at conditions in that environment to form hypotheses as to what might be responsible for their presence.

Other factors to consider in seekimg to use biomarkers as early warning indicators are the ease of observation, the time between establishment of the mark and the death of the marked individual, stability of the populations under study, confounding factors and sampling ease.

I will restrict this presentation to the few biomarkers with which I have had some experience and discuss some of their merits and demerits. These include: Population level observations, e.g. absence of age 0, and Age I year classes; Gross anatomical deformities e.g. curvature of the spine, edema, exopethalmia, gas bubbles in the fins, and gall bladder color and state of fullness; Histological changes, e.g. liver glycogen depletion, oocyte atresia, chloride cell proliferation and apical pitting, and gill hyperthrophy, hyperplasia, and vacuolation; amd Physiological changes, e.g. blood osmolality.

SESSION 2B

MESOCOSMS IN THE STUDY OF FATE AND EFFECTS OF TOXIC CHEMICALS: HOW FAR HAVE WE ADVANCED?/LES MÉSOCOSMES DANS L'ÉTUDE DU DEVENIR ET DES EFFETS DES PRODUITS CHIMIQUES TOXIQUES: OÙ EN SOMMES NOUS RENDUS?

CHAIRPERSON/PRÉSIDENT
Keith Solomon

EFFETS D'UN DÉVERSEMENT DE PÉTROLE EXPÉRIMENTAL SUR LA FLORE BACTÉRIENNE DE L'ESTUAIRE DU SAINT-LAURENT.

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RÉSUMÉ

L'estuaire du Saint-Laurent est une des plus grandes voies navigables au monde et il supporte un important trafic pétrolier tout au long de l'année. Bien qu'aucun déversement pétrolier majeur ne se soit produit jusqu'ici, un tel accident aurait des conséquences écologiques graves sur les écosystèmes du Saint-Laurent. En effet, certaines régions de l'estuaire maritime, particulièrement fragiles, sont considérées comme des zones "à haut risque" en cas de marée noire (Pelletier, 1988). Dans le cadre d'une étude expérimentale menée depuis 3 ans sur le devenir et les effets du pétrole dispersé en eaux froides, nos recherches ont porté entre autres sur la croissance et la capacité d'adaptation de la flore bactérienne du Saint-Laurent en réponse à une contamination élevée ainsi que sur sa capacité à dégrader le pétrole déversé en milieu estuarien.

L'appareillage utilisé, situé à la station aquicole de Pointe-au-Père (Québec), consistait en un groupe de 5 réservoirs en acier inoxidable de 3.5 m^3 chacun (3m de hauteur; 1.4m de diamètre). Une double paroi dans laquelle circulait un liquide réfrigéré ainsi qu'un système de ventilation en surface, permettaient de maintenir en hiver, de très basses températures de l'eau de mer avec formation d'une couche de glace superficielle, simulant ainsi les conditions hivernales rencontrées dans l'estuaire du Saint-Laurent (Pelletier *et al.*, 1989). Des échantillonneurs placés à 1m et 2m de profondeur permettaient l'échantillonnage direct de la colonne d'eau. Plusieurs expériences ayant des durées de 2 semaines à 2 mois, effectuées en été, en hiver et au printemps, ont permis d'étudier la réponse bactérienne à court et moyen termes sous différentes conditions de contamination simulées dans ces mésocosmes (pétrole dispersé chimiquement, non traité ou relargué à partir d'un substrat). Pour chaque expérience, un réservoir non contaminé servait de controle. Des solutions de sels nutritifs étaient régulièrement ajoutées afin de maintenir dans les réservoirs un enrichissement comparable à celui du milieu naturel. Dans chaque réservoir, les bactéries hétérotrophes viables (comptages sur plaques ou par la technique du "Most Probable Number"), les bactéries spécifiques du pétrole (plaques ou MPN) ainsi que les bactéries totales (comptages directs en épifluorescence) étaient régulièrement mesurées.

Des concentrations importantes (de 1 à 70 mg.L^{-1}) de pétrole dispersé ou de produits pétroliers solubles relargués à partir d'un substrat pollué ont été testées. La présence du pétrole induit très rapidement une croissance exponentielle des bactéries hétérotrophes viables. En milieu

non limité en sels nutritifs, la densité bactérienne maximale atteinte dans les mésocosmes est proportionnelle à la quantité de pétrole dispersé dans la colonne d'eau et le taux de croissance augmente avec la température. Par exemple, lors d'une expérience réalisée en été (température moyenne de l'eau de mer: 12.5°C), la densité bactérienne a atteint 2.5 10^6 CFU.mL^{-1}, 8 jours après l'addition de pétrole dispersé chimiquement, à une concentration initiale de 50 mg.L^{-1}. En hiver (temp.: 0°C), pour une gamme de concentrations comparables, la densité bactérienne maximale était atteinte après 3 semaines et était de un à deux ordres de grandeur plus faible. Dans l'eau de mer non contaminée utilisée pour ces expériences, la densité bactérienne variait de 8 10^2 à 3 10^3 CFU.mL^{-1}, selon la saison. Lors d'une expérience hivernale effectuée en conditions extrêmes (temp.: −1.5°C; couche de glace en surface), on a observé, dans les premiers jours de contamination, une poussée des bactéries viables (passant de 10^4 à 10^5 cell.mL^{-1}) alors que le nombre de bactéries totales restait relativement constant, autour de 6 10^5 cell.mL^{-1}. Cette évolution de la flore bactérienne pourrait correspondre à une "phase de réactivation" de la population bactérienne totale, déjà observée par ailleurs, dans des eaux froides soumises à des apports organiques soudains (Delille et Bouvy, 1989). Bien que les bactéries spécifiques du pétrole représentaient quelques fois plus de 10% des bactéries viables quelques jours après l'addition de pétrole, la proportion des bactéries spécifiques ne semble pas toujours être en rapport avec la quantité de pétrole dispersé dans l'eau. Dans les conditions simulées dans ce travail, l'augmentation absolue de la densité des bactéries spécifiques apparait plus adéquate comme critère de la contamination pétrolière de l'eau du Saint-Laurent. En hiver, les bactéries spécifiques ont atteint un maximum de 950 cell.mL^{-1}, après 2 jours d'exposition à une concentration en pétrole de 4.3 mg.L^{-1}, alors que leur niveau moyen dans l'eau non contaminée se situait autour de 45 cell.mL^{-1}, indiquant la potentialité d'adaptation de la population bactérienne indigène en réponse à une contamination pétrolière. Les analyses chromatographiques en phase gazeuse (CPG) faites simultanément sur les résidus de pétrole ont mis en évidence que la croissance des bactéries s'accompagnait d'une biodégradation significative des hydrocarbures (aliphatiques et aromatiques) dispersés dans l'eau, que le pétrole ait été préalablement traité avec un dispersant ou non. Toutefois, la biodégradation était réduite sous de très basses températures de l'eau de mer (<0°C). Dans ces conditions, un léger choc toxique était observé sur les bactéries viables, dans les premiers jours de contamination, confirmant la toxicité potentielle des produits pétroliers sur l'activité bactérienne (Griffiths *et al.*, 1981; Pengerud *et al.*, 1984). La diminution de la diversité spécifique de la population bactérienne a également été notée dans le milieu le plus pollué, et sous des conditions hivernales extrêmes.

Cette étude expérimentale confirme des résultats obtenus en conditions naturelles lors de petits déversements dans l'estuaire du Saint-Laurent (Pelletier *et al.*, 1991; Siron *et al.*, 1991), qui montrent que la biodégradation des hydrocarbures est très variable et dépend étroitement des conditions environnantes. La période la plus critique se situe en hiver, alors que l'activité bactérienne est réduite et que la couverture de glace peut piéger le pétrole en surface et le garder intact, mais dans des conditions favorables (températures plus élevées, apports nutritifs importants, pétrole dispersé), la flore bactérienne du Saint-Laurent semble avoir la capacité de répondre rapidement à un accident pétrolier.

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THE ACCURACY AND PRECISION OF PESTICIDE CONCENTRATIONS FOLLOWING APPLICATION TO LITTORAL ENCLOSURES. M.L. Knuth, U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota.

The use of field mesocosms to study the fate and effects of toxic chemicals has increased in recent years. An acceptable level of accuracy and precision of the concentration of the chemicals being applied to these systems must be attained. For the past six years, The U.S. Environmental Protection Agency, Environmental Research Laboratory - Duluth in cooperation with the University of Wisconsin - Superior, Lake Superior Research Institute, has developed a natural aquatic system testing protocol which utilizes littoral enclosures to assess the environmental impact of pesticides. Littoral enclosures (10 m x 5 m) were constructed in 1986 in a 2 ha pond near Duluth, Minnesota. Each enclosure includes 5 m of natural shoreline and three walls consisting of an inert polyolefin plastic material. The enclosures have an average water depth of 0.6 m, contain an average of 30 m^3 of water, and enclose an average surface area of 47 m^2.

Field studies were conducted using the littoral enclosures with the organophosphorus insecticides chlorpyrifos in 1986, azinphos-methyl in 1990 and 1991, and with the pyrethroid insecticide esfenvalerate in 1988. The objectives of this presentation are to evaluate the accuracy and precision of the aqueous pesticide concentrations. Discussion will focus on the variability of the concentrations between the treatment replicates, the posttreatment time, between applications within the same year and of consecutive years, and between the three pesticides.

All pesticides were commercially available emulsifiable concentrate formulations and were applied to the surface of each enclosure using an 8 L pressurized sprayer. Composite water samples were collected at 1 h posttreatment and at predetermined intervals thereafter until the pesticide could no longer be detected in the water column. All pesticide water samples were liquid-liquid extracted and the extracts were analyzed by gas-liquid chromatography.

The experimental design differed between studies depending on the specific objectives of each experiment. The 1986 chlorpyrifos experiment utilized 12 enclosures with three concentration levels. The 1988 esfenvalerate study utilized 18 enclosures with five levels. The azinphos-methyl study utilized 18 enclosures with four levels in 1990, and two levels in 1991. In addition, all studies contained replicated control enclosures. The accuracy of the pesticide application, defined here as how closely the measured concentrations agreed with the calculated nominal value, was assessed by calculating the measured mean concentration and 95% confidence interval (CI) and comparing them to the nominal value. Precision, defined as how reproducible were the measured concentrations, was assessed by the percent coefficient of variation (CV).

The 5.0 µg/L chlorpyrifos treatment had a measured mean (± SD) maximum concentration of 6.29 ± 1.05 (n=4, 95% CI=4.59-7.94). The first and second treatments (4 wks) of esfenvalerate at a nominal concentration of 5.0 µg/L measured 8.87 ± 4.53 (n=3, -2.37-20.1) and 4.96 ± 2.40 (n=3, -1.00-10.92), respectively. The 1990 and 1991 azinphos-methyl treatments had nominal concentrations of 4.0 µg/L which resulted in measured values of 4.01 ± 1.02 (n=4, 2.47-5.72) and 4.46 ± 1.16 (n=6, 3.25-5.67), respectively.

The variability between replicate enclosures within the same treatment level was calculated for all studies. The mean CV within treatment level, with respect to time for chlorpyrifos ranged from 15.4% to 19.8%. The mean overall CV, including all treatments at all sample times, was 17.7% (n=32). The two applications of esfenvalerate showed mean CV values ranging from 21.2 to 52.8%, and 29.9 to 47.7%, respectively. Overall CV values were 31.7% (n=11) and 39.4% (n=15), respectively. Azinphos-methyl was applied in two consecutive years. In 1990, the within treatment level CV ranged from 17.9 to 37.6% with an overall mean CV of 24% (n=16). In 1991, the CV ranged from 27.7 to 29.3% with an overall mean of 28.5% (n=8).

The measured concentrations of azinphos-methyl were more accurate when compared to the nominal values than chlorpyrifos or esfenvalerate. The Chlorpyrifos study yielded the most precise data, followed by azinphos-methyl; esfenvalerate was the most variable.

The percent CV for all of the littoral enclosure tests at all measurable treatment levels at all sample times ranged from 17.7 to 39.4%. This is more variable than one would expect for physical and chemical properties (pH or dissolved oxygen), but less variable than might be expected for ecosystem structural variables (organism counts). Perhaps more important, the variability is similar to the range of variation typically found for acute and chronic endpoints of single species toxicological tests.

HERBICIDE CONCENTRATIONS IN THE WATER AND SURFACE FILM OF SOME SASKATCHEWAN PONDS.

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Weekly water samples and organic surface film were collected from two small, artificial ponds (dugouts) in southern Saskatchewan during the summers of 1989 and 1990. These ponds represent the only stable surface water supplies in many parts of this region. As such, they are frequented by birds, wildlife and invertebrates, sometimes stocked with fish and used as domestic and agricultural water supplies. Because they are located on cultivated land, the ponds are exposed to pesticide contamination through run-off and atmospheric deposition. This paper describes the measurement of seven commonly used herbicides and compares concentrations found in the surface film with those of the pond water. The herbicides reported are dicamba, MCPA, bromoxynil, 2,4-D, trifluralin, triallate and diclofop.

BIOAVAILABILITY OF SEDIMENT ASSOCIATED DIOXIN CONGENERS TO MUSSEL AND CRAYFISH IN FRESHWATER ENCLOSURES.

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The bioavailability of sediment associated 1,3,6,8-TCDD, 1,3,7,9-TCDD, 1,2,3,4,6,7,8-HpCDD and OCDD was investigated in two 5-m diameter lake enclosures in the littoral zone of a Canadian Shield lake. Sediment-sorbed dioxins were added in June, 1985. The PCDDs reached their maximum concentrations in the sediment by March, 1986. Following this, concentrations decreased with half lives of approx. 4.5 years for the two TCDDs and approx. 6.0 years for the HpCDD and the OCDD. Freshwater mussel (*Anodonta grandis*) and crayfish (*Orconectes virilus*) were caged on the sediment in 1989 and sampled over a period of 103 days. Crayfish generally accumulated more dioxins from the sediment into their tissues than did the mussel. Both species showed higher bioavailability indices for the two TCDDs than for the HpCDD or the OCDD, possibly due to steric factors. The bioavailability of all four congeners varied with the amount of sediment distrubance, whether by lake turnover, dredging or bioturbation, the greater the accumulation of the PCDDs.

MICROBIAL GENE TRANSFER IN RESPONSE TO CHLOROAROMATIC CHEMICAL EXPOSURE: FRESHWATER MESOCOSMS AND INDUSTRIAL MICROCOSMS.

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Bacteria in freshwater and industrial environments are at the foundation of heterotrophic food webs and as such are often the first organisms to respond to chemical contaminants. We have studied phenotypic and genetic responses of specific populations biodegrading chloroaromatic contaminants. Quantitative gene probing techniques have been established that µM concentrations of chlorobenzoates, chloroanaline and chlorobiphenyl can select populations harbouring a mobile genetic element (Plasmid pBRC60 carrying Transposon Tn5271), and that this element transfers horizontally between bacterial populations during selection in freshwater mesocosms. Microcosms containing ground-water and surface water from the Hyde Park industrial landfill were used to select populations capable of mixed contaminant biodegradation. These experiments also demonstrated the importance of genetic adaptation in heterotrophic bacterial communities. The mobile element Tn5271, which is involved in chloroaromatic ring-fussion in these bacteria, was found independently in several unique genetic backgrounds in the industrial strains.

SESSION 2C

METHODS TO DETERMINE FATE AND EFFECTS OF CONTAMINANTS IN LOTIC ENVIRONMENTS/MÉTHODES VISANT À DÉTERMINER LE DEVENIR ET LES EFFETS DES CONTAMINANTS EN MILIEU LOTIQUE

CHAIRPERSON/PRÉSIDENTE
Kristin Day

GROWTH RATES OF UNIONID BIVALVES UPSTREAM AND DOWNSTREAM OF INDUSTRIAL OUTFALLS CONTAMINATED WITH TRACE METALS.

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The St. Lawrence River drains a highly industrialized watershed. In addition to being the outflow of the Great Lakes, the river receives effluents from numerous installations situated on its shorelines which discharge trace metals, xenobiotic organic compounds, and raw sewage. The ecological effects of these discharges are not well known.

Unionid bivalves are abundant throughout the St. Lawrence River, and are potentially informative indicators of biological effects of pollution. Their sedentary, filter-feeding lifestyle lasting 10 - 20 yr amounts to a long-term exposure to dissolved and particulate constituents of their environment. Bivalves are known to be physiologically responsive to a variety of pollutants, which can result in the impairment of such processes as growth. Pattern and rate of growth may thus be related to pollution-induced stress.

Natural populations of the unionid Lampsilis radiata were sampled upstream and downstream of four industrialized areas that discharge effluents containing trace metals and other contaminants into the upper St. Lawrence River : Cornwall - north channel; Cornwall - south channel; Montreal; and Sorel. Sediments downstream of these outfalls contain elevated amounts of Cd, Co, Cu, Cr, Pb, Hg, Ni and/or Zn relative to the sediments upstream of the outfalls. The downstream site at Cornwall - south channel is also contaminated with polychlorinated biphenyls (PCBs). For approximately 50 clams from each of the 8 collection sites, external annual growth rings of the shells were measured for length. Successive annual lengths were then fitted by a Von Bertalanffy equation using the Walford Plot technique (McCuaig and Green 1983) to estimate the pattern of growth for each individual. Pairwise comparisons were made between upstream and downstream groups by examining intercept of the Walford Plot regression (length after first year of growth), slope of Walford Plot regression (rate of decrease in yearly increments in length), and final asymptotic size (maximum length).

Comparisons between upstream and downstream sites within each pair show higher mean slope for the two downstream sites in the Cornwall area. No difference in slope between upstream and downstream populations at Sorel was detected, but for the Montreal site the upstream population shows higher slope. Mean intercept of Walford Plots for the downstream Cornwall - north channel population was lower than that for the upstream site, indicating lower length after one year's growth; differences between sites in the Cornwall - south channel and Sorel areas were not significant; and at Montreal the upstream population had much lower mean intercept. Bivariate plots of 95% confidence ellipses around the mean intercept vs mean slope for the Walford Plot regressions (Fig. 1) show that for each pair of sites the upstream and downstream populations are distinct. Estimated growth curves based on the Von Bertalanffy model for the eight populations are shown in Fig. 2. Final asymptotic sizes are lower for the downstream populations at Sorel; not different between upstream and downstream sites at Cornwall - s. channel; and higher for the downstream population at Cornwall - n. channel and Montreal. Thus for 3 out of the 4 site pairs, some aspect of mean growth was depressed in the downstream clams relative to those from upstream.

Upstream and downstream sites in the Cornwall (north and south channels) and the Sorel areas do not differ greatly from each other in chemical (specific conductance, alkalinity, dissolved calcium concentration) or physical (current speed, sediment particle size distribution) conditions that are known to affect clam growth, except for concentrations of several trace metals and PCBs in sediments. Depressions of shell growth in the downstream populations are therefore best explained by toxicity due to certain or several trace metals and/or (in the south channel at Cornwall) PCBs. Such effects have been observed in other bivalve species. Excessive amounts of metal ions in tissues can impair or inhibit function of a variety of enzymatic procesess, leading to sublethal effects such as reduced growth of shells (Langston 1990).

Clams from the upstream site in the Montreal area show markedly lower rates of growth and estimated final length copmpared to those from the downstream site. Located near the junction of the Ottawa River, alkalinity, dissolved calcium concentration and specific conductance of the upstream site were all roughly less than one third of the values for the mainstream of the St. Lawrence River. Slower growth and smaller size in these clams are thus likely due to the influence of the softer water of the Ottawa River, as was observed by Magnin and Stanczykowska (1971).

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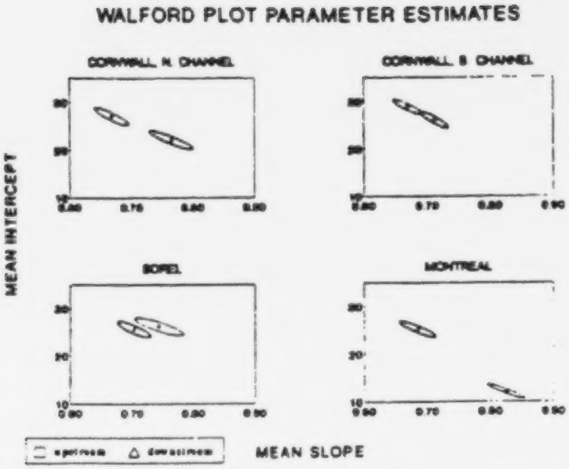
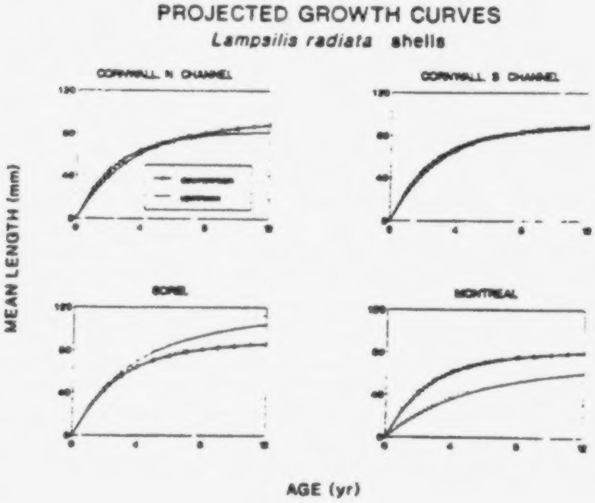


1. 95% confidence ellipses around mean intercept vs mean slope from Walford Plot regressions using external annual ring lengths of Lampsilis radiata. Clams were collected from paired study sites located upstream and downstream of four industrialized areas in the upper St. Lawrence River.



2. Estimated mean growth curve (with 95% confidence bands) for each of eight sampled populations of Lampsilis radiata, based on Walford Plot analyses of external growth rings and the von Bertalanffy model. Final asymptotic sizes are approached as the curves level out.

THE EFFECT OF EDTA ON CADMIUM AND ZINC UPTAKE AND TOXICITY IN THE SUBMERGED AQUATIC MACROPHYTE *Lemna trisulca* L.

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The chelator ethylenediaminetetraacetic acid (EDTA) is often included in nutrient media to facilitate the uptake of iron by aquatic macrophytes. To optimize growth in *Lemna*, Landolt and Kandeler (1987) recommend the consistent use of EDTA in nutrient media. The chelator EDTA will also bind metals other than iron, even though the EDTA binding constant for Fe is the highest of all metals (Skoog and West, 1969). As metal toxicity is thought to be related to free ion activity and not total concentration (Borgmann, 1983), the addition of EDTA may potentially confound the measurement of metal toxicity due to chelator-metal interactions.

Results on the effect of EDTA and other chelators on the toxicity of metals in aquatic macrophytes are sparse and contradictory. Nor and Cheng (1986) showed that Cu uptake by *Eichomia crassipes* could be prevented by excess EDTA, while Nasu et al.(1983) found that Cu, but not Cd toxicity, was antagonized by EDTA in *Lemna paucicostata*. Polar and Kucukcezzar (1986) also found chelators ineffective in ameliorating Cd toxicity in *Lemna gibba*, but Kwan and Smith (1991) found that 50 µM EDTA almost completely prevented Cd uptake in *Lemna minor*. Recognizing that chelators may affect metal uptake and toxicity, the U.S. EPA published a bioassay protocol entitled, "*Lemna* acute toxicity test" (U.S. EPA, 1985) which specifically excluded chelators. The purpose of our study was to examine the effect of EDTA on Cd and Zn uptake and toxicity in *L. trisulca* and to determine if the protocol for the *Lemna* acute toxicity test is appropriate.

Lemna trisulca was grown using sterile culture techniques in 1L erlenmeyer flasks containing 750 mL of a complete, filter-sterilized nutrient medium (Huebert et al., 1990). The cultures were aerated to maximize growth and the medium was replaced daily at an exponentially increasing rate that followed the growth rate of control treatments. Cadmium or Zn was added to the cultures and the EDTA:Fe ratios were varied by changing either the EDTA or Fe concentration. The plants were grown for two weeks, after which the fronds were counted and the multiplication rate (MR) calculated as MR = 1000 ($\log F_1$ - $\log F_0$)/t where F_0 and F_1 are the initial and final frond numbers, respectively, and t is the culture time in days. Cadmium and Zn analyses were carried out by atomic absorption spectroscopy on acid digested samples.

Control treatments contained no Cd, 0.08 µM Zn and an EDTA:Fe ratio of 1:1 at 9 µM. Cadmium, when increased to 0.32 or 1.28 µM, reduced the MR of L. trisulca by 35 and 61%, respectively. Removing the EDTA lowered the MR of L. trisulca 28% and produced stunted and chlorotic fronds, but the relative effect of Cd was not altered. The inhibitory effect of Cd on MR was completely reversed when the EDTA:Fe ratio was increased to 4:1, either by an increased EDTA, or decreased Fe concentration. Increasing the EDTA:Fe ratio to 16:1 at 144 µM EDTA reduced the MR 17% but still prevented Cd toxicity.

L. trisulca accumulated 620 µg Cd/g dry-weight at 0.32 µM Cd and 2300 to 3400 µg Cd/g dry-weight at 1.28 µM Cd. When the EDTA:Fe ratio was increased to 4:1, accumulation of Cd decreased by approximately 98% at both 0.32 and 1.28 µM Cd.

Zinc at a concentration of 6.12 µM decreased the MR 28% and at 24.5 µM almost completely inhibited growth. The effect of EDTA on Zn uptake and toxicity was less pronounced than on Cd, probably due to the fact that the Zn concentrations were higher. The inhibitory effect of 6.12 µM Zn was eliminated by increasing the EDTA concentration to 36 µM but only at 144 µM EDTA and a 16:1 EDTA:Fe ratio was the toxicity of 24.5 µM Zn completely neutralized.

L. trisulca accumulated approximately 8.7 mg Zn/g dry-weight at 6.12 µM and 20 to 30 mg Zn/g dry-weight at 24.5 µM. When the EDTA:Fe ratio was increased to 16:1 at 144 µM EDTA, accumulation of Zn was reduced by approximately 96% at 6.12 µM and 99% at 24.5 µM .

Our results indicated that EDTA strongly antagonized Cd and Zn uptake and toxicity when the available EDTA was in excess of the metal concentration. This suggests that it was the free ion activity and not the total metal concentration which determined uptake and toxicity. The proportion of EDTA available for chelating Cd and Zn was the amount of EDTA in excess of the Fe concentration. The poor condition of L. trisulca grown with no chelator in the medium indicated that it is inappropriate to culture Lemna without a chelator as outlined by the U. S. EPA (1985).

Two strategies may effectively deal with the chelator requirements of aquatic macrophytes without confounding the measurement of the uptake and toxicity of metals. Firstly, one can establish a 1:1 EDTA:Fe ratio and add the minimum of EDTA that will support maximal growth rates. This is the strategy used in our studies (Huebert and Shay,1991a; 1991b) and it appears to be effective in that the plants grow rapidly while still being sensitive to metal toxicants. The problem with this strategy is that EDTA is unstable in light (Lockhart and Blakeley, 1975) and Fe uptake occurs, so that there are continual changes in the chelating properties of the medium. It is imperative that replacement of media take place to try to minimize these changes. The second strategy is to maintain a high, excess chelator concentration so that changes in metal chelation will be minimized. This approach requires a speciation program in order to calculate free ion activities, data which are difficult to obtain and of uncertain value without precisely defined and maintained conditions. High EDTA concentrations in the 100 μM range may also be inhibitory, as was found in our studies with L. trisulca.

When measuring metal toxicity, chelators must be included, and chelator:iron ratios must be carefully considered and defined, because of the poor growth of plants grown in media lacking EDTA and the strong antagonistic interaction of available EDTA on metal toxicity. The U. S. EPA protocol for toxicity assessment in Lemna should be changed to include chelators at a defined chelator:iron ratio in order to reflect the growth requirements of Lemna and to prevent results from being confounded by variable chelator-metal interactions.

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PCB DYNAMICS IN A SOUTHWESTERN ONTARIO CREEK.

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INTRODUCTION

Polychlorinated bipyhenyls (PCBs) are a group of ubiquitous, man- made chemicals that, through atmospheric deposition (Eisenreich et. al., 1981; Patton et al., 1989), have spread throughout aquatic environments resulting in contamination of biota, sediments and vegetation. In the early 1980's, PCBs were discovered in yearling common shiners collected from Pottersburg Creek, London, Ontario. Further monitoring revealed that much of the creek sediments were contaminated with PCBs resembling mixtures of Aroclor 1248, 1254, and 1260. Remediation efforts between 1985 and 1986 included the removal of sediments from the most contaminated areas of the creek which were identified as being between Clarke Sideroad and Trafalgar Street as well as Walkers Drain (Fig. 1). However, in the fall of 1987, subsequent monitoring of the remediated areas revealed that the sediments had become recontaminated.

Following the discovery of the extent of recontamination, several initiatives were undertaken. These included 1) the removal of more sediment; 2) cleaning the storm sewer network below the source of PCBs; 3) implementing a water and sediment monitoring program ; 4) establishing the present study. The objectives of the present study are to 1) determine what factors account for the current PCB concentrations in biota and 2) to determine when PCB concentrations in biota are expected to decline to acceptable levels.

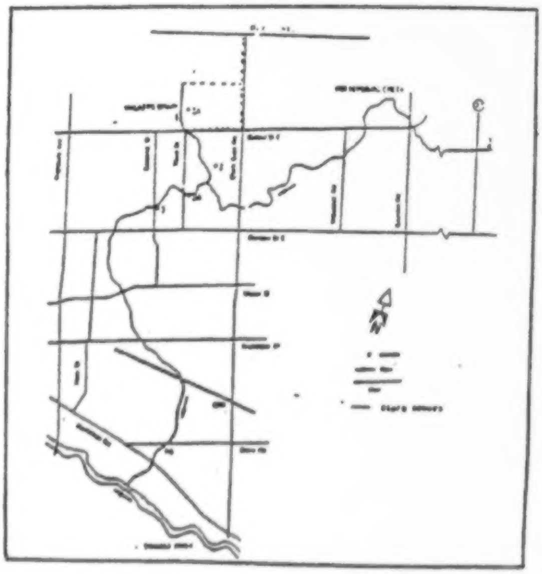

Figure 1. Location of monitoring stations along Pottersburg Creek, Walker Drain and the Thames River, in London, Ontario.

Bioaccumulation of PCBs may occur via bioconcentration (i.e. uptake from water) or biomagnification (the uptake of a chemical through ingestion). Previous studies have emphasized bioconcentration as being the mechanism responsible for contaminant levels in biota (Schneider 1982; Duursma et al. 1989). Bioconcentration states that the concentration in an aquatic organism can be attributed to the chemical concentration in the water, the octanol/water partition coefficient, and the lipid fraction of the organism. However, many experiments have shown that aquatic organisms are capable of bioaccumulating PCB's above concentrations which would be expected from water alone (Oliver and Niimi 1988; Gobas et al. 1987) and that variation in lipid content does not adequately explain the departure of this relationship. This would suggest that some other mechanism, such as biomagnification, is responsible for the contaminant levels in aquatic biota, especially those occupying the top of food chains (Thomann 1987; Rasmussen et al. 1990).

Chemical classification of PCBs by K_{ow} (ocatanol/water partition coefficient) can aid in the selection of the appropriate mechanism governing contaminant levels in biota. For those PCBs that have a log K_{ow} of less than 5, food chain accumulation is generally small, henceforth, bioconcentration or simple equilibrium partitioning of contaminants may sufficiently explain contaminant levels in biota. For those PCBs who have a log K_{ow} of more than 7, there is uncertainty as to the actual significance of food chain transfers. It is believed that these substances are quickly absorbed or adsorbed by biota or organic matter and remain locked in place due to reduced transfer efficiencies. For those PCBs who have a log K_{ow} between 5-7, a food chain model is needed for estimating contaminant levels in biota.

The discovery of PCBs in Pottersburg Creek has provided an opportunity to gain a better understanding of their movement in aquatic ecosystems and the mechanism of PCB transfer to higher trophic levels. Moreover, the three Aroclors found in Pottersburg Creek have a log K_{ow} of between 5.8 and 7.5, consequently, the direction of this study was towards the synthesis of a food web in order to explain contaminant levels in biota. If the biota show an increase in PCB body burden with increasing trophic status beyond that which can be explained by variation in lipid content, then one could conclude that PCBs biomagnify through aquatic food webs resulting in highest accumulations in top predators. In addition to the above, the ultimate objective of this project is to develop a model in order to predict the time required for PCB levels in aquatic biota to reach the IJC (1975) aquatic life protection guideline of 100 ng/g.

MATERIALS AND METHODS

Collection and analyses of stream components, 1988 - 1991.

Samples of algae, aquatic macrophytes, various macroinvertebrates and fish were collected for biomass and PCB analysis at 4 sites along Pottersburg Creek (Fig.1). Water and grab samples of sediment were also collected. Station 1 was used as a reference site and Stations 2, 3 and 4 were located consecutively downstream of the point source of PCBs.

Invertebrates gathered for biomass and density determination were collected with a T-sampler enclosing an area of $0.05m^2$, and sieved through a 300 μm bag. Invertebrates were sorted live from the debris in white enamel trays and subsequently preserved in 70% ethanol for later identification and enumeration. All organisms were identified to the lowest taxonomic level, usually genus, except for oligochaetes which were identified to species.

Invertebrates gathered for PCB analysis were collected and sorted live in white enamel trays until an adequate weight was obtained for analysis (i.e. 3g or more). Animals were separated into their respective classes (e.g. oligochaetes, chironomids, leeches) and weighed to the nearest 0.01g. Samples were subsequently wrapped in hexane-rinsed aluminum foil, placed in plastic Whirl-Pak bags and kept frozen until analyzed. The samples were analyzed for total PCBs and lipid with results being reported on a wet-weight basis. Analyses were carried out at the Ontario Ministry of the Environment's laboratory in Rexdale using packed column gas chromatography (OMOE, 1986).

Construction of a Food Web

Fish were collected with a seine net from Stations 1,2,3 and 4 and preserved in 20% formalin. Collections were made in the summer and winter of 1988 and the summer of 1989. Fish stomachs were dissected and a list of food items was compiled. A food web of Pottersburg Creek was synthesized from feeding observations and gut contents analysis. Points of PCB entry and loses were also identified.

Determination of PCB uptake and depuration rates

Uptake experiments were set up for the following biota: crayfish, leeches, fish, and oligochaetes. Crayfish (*Orconectes* sp.) were captured in the fall of 1990 from the Eramosa River, Guelph, Ontario. A total of 36 crayfish were placed in 6 cylindrical wire cages which were 45 cm long and had a diameter of 20cm. Leeches (*Nephelopsis obscura*) were purchased from a local bait dealer. Approximately 36 leeches were placed in each of 3 wooden cages measuring 45cm X 30cm X 20cm. Two opposite sides of each cage had 6cm X 18cm holes which were covered with a 300 μm mesh screen to facilitate water flow. The tops of two of the cages were covered with 300 μm mesh screen while the other cage was covered with a 2mm mesh fibreglass screen. The first cage contained contaminated sediments collected from Station 2. The second cage contained contaminated sediments from station 2 which had been previously frozen to kill any indigenous organisms and the third cage contained only clean rocks. Oligochaetes (*Lumbriculus variegatus*) were purchased from a local pet store and approximately 100 grams of worms were placed in each of 2 wooden cages which had the same dimensions as those described above for leeches. The top of each cage was covered with a 300 μm mesh screen while the sides were covered with 100 μm mesh screens. The first cage contained contaminated sediment collected from Station 2 which had been previously frozen and the second cage contained only clean rocks. Fish (*Pimephales promelas*) were collected from Dorsett Ontario and approximately 400 fish were placed in a wire circular cage which was approximately 2m in diameter and whose mesh size was 0.63 cm. The bottom of the cage was left open so the fish were exposed to creek sediments, vegetation and food.

The cages containing the biota were anchored to the stream bed with rocks at the contaminated site (Station 2) and subsequently collected after the following days: 1) day 8, 15, 23, 36, 50, 58, 71 for crayfish; 2) day 8, 11, 16, 29, 51, 85 for leeches; 3) day 7, 10, 16, 29, 43, 56 for worms; 4) day 10, 12, 20, 35 for fish.

Depuration experiments were conducted for the following biota: fish, crayfish and worms. Fish and crayfish were collected from contaminated areas of the creek (Station 2 and 3 respectively) and placed in 45 L aquaria. The water was changed every 4 days to prevent PCB accumulation. Worms were collected from Station 2 and placed in 10 gallon aquaria which contained silica sand. Fish and crayfish were collected after 4, 6, 10, 18, 34, and 66 days. Worms were collected after 2, 5, 11, 23, 35 and 63 days. Each group of animals was weighed live to the nearest 0.01g, wrapped in hexane-rinsed aluminum foil, placed in Whirl-Pak plastic bags, and frozen until analyzed. Replicate samples were submitted for each time period where possible. In addition, samples of all groups of animals were submitted for PCB analysis prior to the start of the depuration experiments.

RESULTS AND DISCUSSION

Food web relationships

The food webs of Station 2 and 3 (Fig. 2 and 3) were found to be similair in species composition, however, quantitatively they differed with respect to biomass. Crayfish and chironomids dominated Station 3 while worms were the dominant invertebrates found at Station 2. Sediment type and vegetation also differed between the two stations. Station 2 sediment is typically a fine silt which is 15 - 30 cm deep at places. In the summer months, *Cladophora* is dominant throughout this area with some *Potamageton* growing along the edges of the creek. These overgrowths of algae die off in the late fall and are subsequently carried downstream. Station 3 is characterized by a shallow layer of sand and gravel overlaying hard compact clay. *Cladophora* and *Potamageton* are present but in lesser quantities. Station 4 had a greater diversity of habitat than Station 2 and 3: large riffles, pools and under-cut banks were evident. The sediments consisted of large rocks, gravel and sand overlaying compact clay.

PCB concentrations in sediments from Station 2, 3 and 4 decreased temporally as well as spatially from 1988 to 1990. In 1988, Station 2 sediments contained 4130 ng/g (dry weight) PCB while 1990 sediments contained 885 ng/g. PCB concentrations at Station 3 have decreased from 920 ng/g to 63 ng/g while concentrations at Station 4 have dropped from 60 ng/g to 30 ng/g. Presently, PCB concentrations in sediments remain above 500ng/g at Station 2 while concentrations at the other stations are much lower, i.e. less than 100 ng/g, for most of the year.

Even though the initial source of PCBs into Pottersburg Creek has been alleviated, the sediments remain a continuing source to biota and vegetation. PCBs bound to sediments may be redistributed to the water by scouring of bottom sediments caused by catastrophic drift, bioturbation and desorption processes thus making them more available to biota and aquatic plants (Klump et al. 1987; Karickhoff and Morris 1985). In 1990, PCB concentrations in water were below detection limits (<20 ng/L) for most of the year, except after heavy rainfall events when concentrations reached 330 ng/L at Station 2.

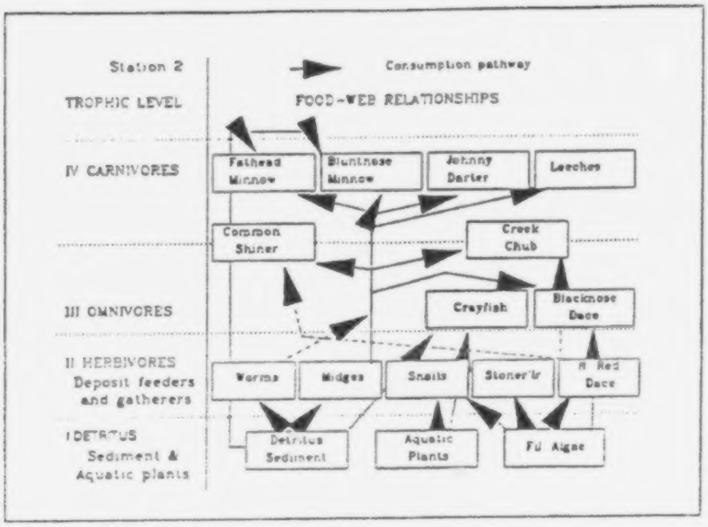
Station 2
Consumption pathway
TROPHIC LEVEL
FOOD-WEB RELATIONSHIPS
IV CARNIVORES
Fathead Minnow
Bluntnose Minnow
Johnny Darter
Leeches
Common Shiner
Creek Chub
III OMNIVORES
Crayfish
Blacknose Dace
II HERBIVORES
Deposit feeders and gatherers
Worms
Midges
Snails
Stoner'lr
N Red Dace
I DETRITUS
Sediment & Aquatic plants
Detritus Sediment
Aquatic Plants
Fil Algae

Figure 2. Food web of Station 2 outlining major predator - prey relationships.

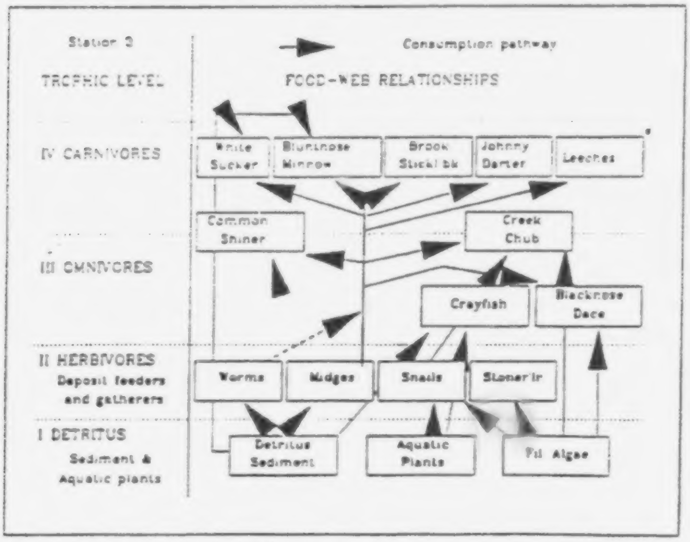
Station 3
Consumption pathway
TROPHIC LEVEL
FOOD-WEB RELATIONSHIPS
IV CARNIVORES
White Sucker
Bluntnose Minnow
Brook Stickl bk
Johnny Darter
Leeches
Common Shiner
Creek Chub
III OMNIVORES
Crayfish
Blacknose Dace
II HERBIVORES
Deposit feeders and gatherers
Worms
Midges
Snails
Stoner'lr
I DETRITUS
Sediment & Aquatic plants
Detritus Sediment
Aquatic Plants
Fil Algae

Figure 3. Food web of Station 3 outlining major predator - prey relationships.

The uptake of chlorinated hydrocarbons by phytoplankton (Biggs et al. 1980; Sodergren and Gelin 1983) aquatic macrophytes (Strek and Weber 1982) and filamentous algae, e.g. *Cladophora*, (Mowrer et al. 1982; Larsson 1987) is rapid and initially follows first order kinetics (Larsson 1987). Thus, the partitioning process governs PCB uptake and in turn is dependent on the concentrations of PCBs in the water (Larsson 1987). In 1988, algae and aquatic macrophytes collected at Station 2 were able to accumulate substantial quantities of PCBs, 150 ng/g and 770 ng/g, respectively. In 1990, PCB concentrations in *Cladophora* declined to 76 ng/g while PCB concentrations in *Potamageton* were non-detectable. No PCBs were detected in algae and aquatic macrophytes at Station 3. Filamentous algae (*Cladophora*) and aquatic macrophytes (*Potamageton*) are consumed by scrapers, i.e. snails and shredders,i.e. crayfish. Algae are also consumed by a variety of fish in Pottersburg Creek. Analysis of gut contents has revealed that filamentous algae are the primary diet of the northern redbelly dace (*Chrosomus eos*) and the stoneroller (*Campostoma anomalum*). Common shiners (*Notropis cornutus*) and blacknose dace (*Rhinichthys atratulus*) also consume filamentous algae but to a lesser extent. As no metabolism or elimination of PCBs has been recorded in algae (Hutzinger et al. 1974), the rapid adsorption and uptake of PCBs by algae and aquatic macrophytes constitute a means for the introduction of these compounds into food webs (Mahanty 1986).

Algae and aquatic macrophytes are a food source for snails and crayfish. In addition, crayfish eat detritus, and other organisms (Hynes 1970), which in turn are known to accumulate or sorb PCBs. In 1988, *Helisoma sp.* collected from Station 3 had PCB residues of 360 ng/g and young of the year crayfish from Stations 2, 3 and 4 had residues of 960 ng/g, 720 ng/g and 320 ng/g, respectively. Decreasing body burdens in crayfish indicate a reduction of PCB availability with increasing distance from Walkers Drain (Stn. 2). Older crayfish from Station 3 were noted to accumulate 40% more PCBs than young of the year. This would suggest that length of exposure, coupled with higher lipid levels in the older individuals, may have an effect on PCB bioaccumulation. In 1989 and 1990, snails collected at Station 3 showed no accumulation of PCBs, however, snails collected from Station 2 in 1990 showed accumulations of 280 ng/g. PCB residues in crayfish collected from Station 2, 3 and 4 varied depending on the size of the animal as well as the lipid content albeit, concentrations were noted to be lower than in the previous year.

Crayfish and snails are eaten by a variety of fish (Scott & Crossman 1972). Gut contents analysis confirmed the ingestion of snails by creek chub (*Semotilus atromaculatus*) in Pottersburg Creek (Station 3), however no crayfish were found in the stomachs of the fish. Therefore, snails may contribute to the overall PCB body burden of fish such as creek chub.

The sediments remain a continuing source of PCBs to chironomids and worms. Ingestion of sediment (Roberts and Meier 1982) and PCB contaminated organic particles after they settle on the sediment (Landrum and Scavia 1983; Oliver 1984) by these organisms has contributed to their body burdens. Meier and Rediske (1984) have been able to show that larvae of the midge *Glypotendipes barbipes* can accumulate significant concentrations of PCBs from substrate feeding alone. Initial bioaccumulation by midges has been shown to be very rapid with steady state concentrations being reached in 4 to 5 days (Swindoll & Applehans 1987). While uptake is very rapid, PCBs have been shown to be poorly eliminated from benthic organisms (Swindoll & Applehans 1987; Meier & Rediske 1984). Thus, chironomids and worms serve as major vectors for the transfer of PCBS to higher trophic levels.

PCB residues in oligochaetes and chironomids were among the highest for 1988 biota, reaching concentrations of 3030 ng/g and 1890 ng/g respectively in the summer months. Residues in 1990 decreased to less than 1000 ng/g for worms and less than 500 ng/g for chironomids.

Gut contents analysis of fish and feeding observations revealed that chironomids and worms are major food items of all fish species except the northern redbelly dace and the stoneroller who feed primarily on algae. These findings are in agreement with Gilliam et al., (1989) who showed that fish such as creek chub consume worms. In addition, leeches are also predators of chironomids and worms. Leeches (*Erpobdella punctata*) captured from Pottersburg Creek were observed eating chironomids in captivity. As well, several authors have documented that the main food items of leeches are oligochaetes and chironomids (Davies et al 1981; Anholt 1986). The decrease of PCBs in leeches mirrors the decrease in their prey whereas sediment concentrations have remained constant for these areas of the creek.

Leeches were found to occupy the same trophic level as fish consequently PCB concentrations in leeches approached and at times exceeded those concentrations found in fish. In the summer of 1988, PCB residues in leeches were 2000 ng/g at Station 2 and 1060 ng/g at Station 3. In 1989, residues dropped to 1100 ng/g at Station 2 and 700 ng/g at Station 3. Leeches are preyed upon by a variety of fish (Scott & Crossman 1973) but were not found to be major food items of the fish found at Station 2 and 3 in Pottersburg Creek.

The ten species of fish that have been identified from Pottersburg Creek can be grouped into four categories based on their primary diet: algae grazers [stoneroller (*Campostoma anomalum*), northern redbelly dace (*Chrosomus eos*)]; chironomid feeders [brook stickleback (*Culaea inconstans*), johnny darter (*Etheostoma nigrum*)]; bottom ooze feeders [white suker (*Catostomus commersoni*), bluntnose minnow (*Pimephales notatus*), fathead minnow (*Pimephales promelas*)]; and general feeders [common shiner (*Notropis cornutus*), creek chub (*Semotilus atromaculatus*), blacknose dace (*Rhinichthys atratulus*)]. It is believed that food selection and habitat utilization will have an effect on PCB accumulation in these four groups of fish. PCB concentrations are believed to be the highest in bottom ooze feeders followed by the chironomid feeders, the generalists and finally the algae grazers.

Common shiners collected in July 1988 had lower PCB residues than johnny darters collected from the same stations i.e. (Station 2 shiner - 1960 ng/g, darter - 2780ng/g). The observed differences can be attributed to the position of the fish in the water column as well as their diet. Johnny darters were found to feed primarily on chironomids which themselves exhibited high PCB levels. On the other hand, analysis of common shiner fish stomachs revealed no preferred diet. However, it was noted that in the spring and early summer, their diet consisted primarily of surface insects. Gilbert (1964) found that common shiners usually feed off the surface but will take food off the bottom. In the fall and winter months, the common shiner switches its diet to detritus, sediment, algae or invertebrates depending on their respective availabilities. Thus, depending on the time of year, PCB residues in common shiners may vary depending on their diet.

PCB concentrations in common shiners and johnny darters varied in 1989 depending on station location and lipid content. Moreover, it was noted that PCB concentrations in both species of fish did not decrease over the two year period perhaps as a result of biomagnification. In 1990, PCB concentrations in common shiners collected from most areas of the creek have decreased, however, residues in fish closest to the point source of contamination remain elevated (2100 ng/g). PCB concentrations in the northern redbelly dace, collected from Station 2 in 1990, were as high as 2200 ng/g while concentrations in the blacknose dace ranged between 1400 to 3050 ng/g. PCB concentrations in the stoneroller, collected from Station 3 in 1990, reached 500 ng/g and 580 ng/g in creek chub.

Bioaccumulation of PCBs

The bioconcentration concept suggests that uptake of PCBs is dependant on the log K_{ow} of a chemical and the lipid fraction of an organism. If biota from Pottersburg Creek are indeed uptaking PCBs from the water column then each organism having the same lipid composition and proportion should bioaccumulate the same amounts of PCBs, regardless of their position in the food web (trophic level). The biomagnification concept suggests PCB residues in biota are attributed to the ingestion of contaminated prey which subsequently leads to higher PCB concentrations in predators. Therefore, if uptake of PCBs is through ingestion then biota should accumulate different concentrations of PCBs depending on their position in the food web.

In Pottersburg Creek, PCB concentrations in biota from Station 2 tended to be greater than those at Station 3 and these in turn were greater than concentrations at Station 4 (figure 4), but, PCB concentrations were not significantly different between sites, $p = 0.07$, probably because of limited data. However, the relationship between PCB concentration and lipid was similar between sites, .i.e. the slopes of the lines were equal. Consequently, PCB concentrations in biota appear to be decreasing along the length of the creek away from the point source.

The relationship between PCBs and lipid for biota from Station 2 and 3 (Figure 5 and 6) suggests that organisms tend to accumulate PCBs relative to their position in the food web. Fish and leeches occupying the top of the food web, tended to accumulate more PCBs than the lower trophic level organisms. Crayfish in turn accumulated more PCBs than did chironomids at Station 3 and worms at Station 2. Because all data are not yet available, the slope of the lines in Figure 5 and 6 were extrapolated from Figure 4 and assumed equal for all biota. As more data become available, the actual slopes of the lines will be deduced, followed by statistical analysis of the data.

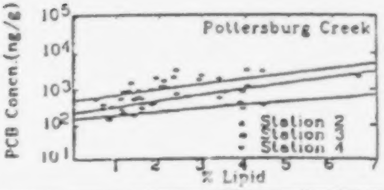

Figure 4. Relationship between PCBs and lipid at different Stations in Pottersburg Creek.

Model: PCB = Station * Lipid
R^2 =0.57 P=.0001

	F value	P
Station	2.81	0.07
Lipid	12.08	0.001
Slope	0.19	0.83

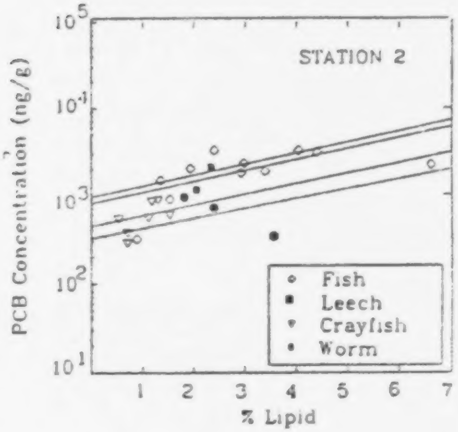

Figure 5. Relationship between PCBs and lipid for biota from different trophic levels at Station 2.

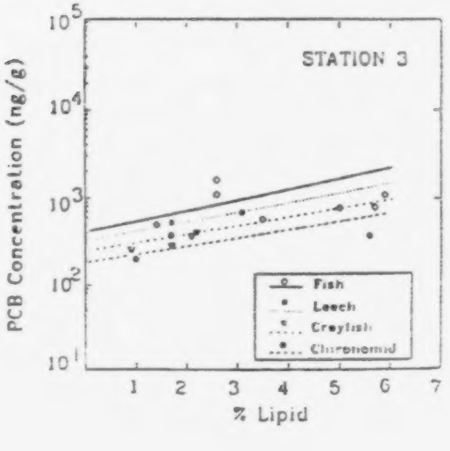

Figure 6. Relationship between PCBs and lipid for biota from different trophic levels at Station 3.

Uptake and depuration

Uptake and depuration experiments are presently being conducted for fish, crayfish, leeches and worms. Results to date indicate that uptake of PCBs is rapid (Figure 7) however, concentrations were noted to be lower than those from collected biota from the same station (leeches, Stn.2 - 1100 ng/). Lower accumulations are attributed to the short time interval of the experiment, i.e., 85 days. In addition, uptake rates were higher in leeches exposed to creek water, sediments and contaminated food (eg. 500 ng/g lipid in 51 days) compared to leeches only exposed to creek water (eg. 133 ng/g lipid in 51 days) suggesting that biomagnification may be the primary mechanism governing contaminant levels in biota.

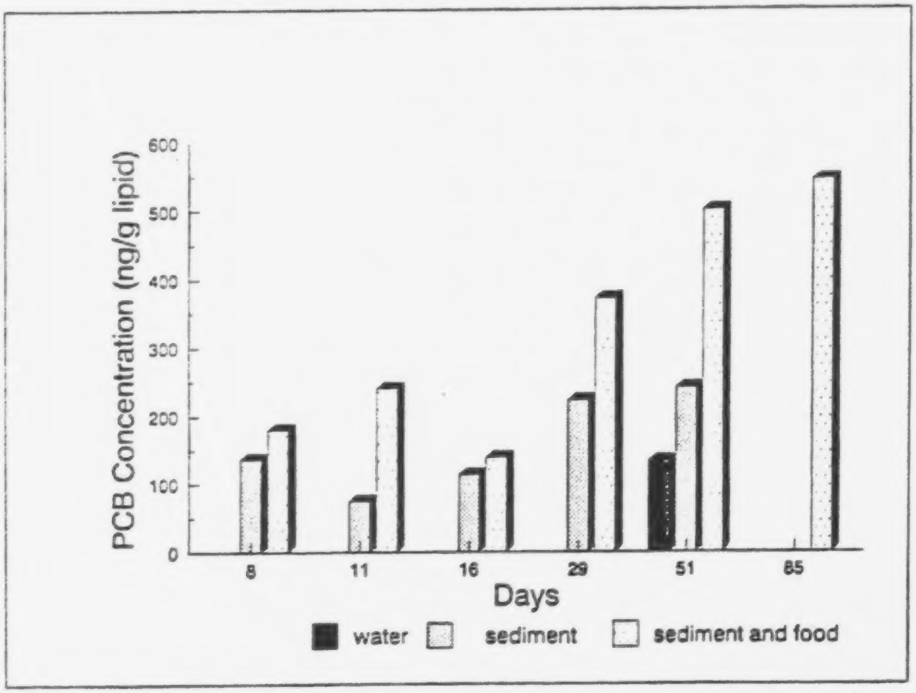

Figure 7. Uptake of PCBs by leeches

Modelling

Upon the completion of the uptake and depuration experiments, all available information and data collected from Pottersburg Creek will be integrated and placed into an ecological framework. PCB transfer rates between the different components of the stream (i.e. fish, invertebrates, water, plants, etc.) will be deduced mathematically and subsequently incorporated into a simulation model in order to determine when PCB concentrations in biota are expected to decline to acceptable levels.

CONCLUSION

As reflected in the data, PCB concentrations in biota, water and sediments from Pottersburg Creek appear to be decreasing with time as well as along the length of the creek away from the point source. However, PCB residues in aquatic biota still remain above the IJC aquatic life protection guideline of 100 ng/g with fish showing the highest concentrations.

From within the creek, the sediments remain a continuing source of PCBs to biota. Scouring of bottom sediments caused by catastrophic drift, bioturbation and desorption processes continuously redistribute PCBs making them more available to aquatic biota. Aquatic plants quickly sorb PCBs from the water and in turn are a food source to snails and crayfish. Chironomids and worms ingest contaminated sediment and organic matter after it settles out on the sediment and are a major food source of fish and leeches. This movement of PCBs up the food web consequently results in highest accumulations in leeches and fish as reflected in Figure 5 and 6, suggesting that biomagnification may be the mechanism responsible for contaminant levels in biota.

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QUANTIFYING CONTAMINANT DYNAMICS IN THE HURON - ERIE CORRIDOR.

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By deploying biomonitors (Elliptio complanata) at the mouth of the St. Clair River and the headwaters of the Detroit River and estimate the bioavailable chemical loadings were made. The fate of bioavailable chemical in the Lake St. Clair food web was quantified by determining fugacity ratios for organisms representative of different trophic levels and feeding strategies. Relatively large and species specific fugacity ratios indicate that food web dynamics can regulate chemical levels and distributions in food webs.

UTILISATION DE LA STERNE PIERREGARIN COMME SONDE BIOANAYLTIQUE DES NIVEAUX DE CONTAMINATION PAR LES ORGANOCHLORÉS ET LES CHLOROPHÉNOLS DANS LE SYSTEME DU SAINT-LAURENT.

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La sterne pierregarin a été utilisée comme bioindicateur des niveaux de contamination en milieu dulcicole (Valleyfield) et marin (Carlton, Lles-de-la-Madeleine et Gaspésie) pour les pesticides organochlorés, les biphényles polychlorés et certains chlorophénols. Les oeufs, les foies et les reins ont été analysés par extraction organique et chromatographie en phase gazeuze (GC-ECD). Les premiers résultats d'analyses montrent qu'il semblerait exister un patron général de distribution des contaminants. La colonie de Valleyfield possède les plus fortes concentrations (max: 1,63 ppm et 1,27 ppm de p,p'-DDE dans les oeufs et dans les foies d'adultes), les plus basses concentrations étant aux Iles-de-la-Madeleine (min: $2{,}5 \times 10^{-3}$ ppm de mirex dans les foies d'adultes). Ce patron est valable pour les contaminants plus importants parmi les pesticides (p,p'-DDE, p,p'-DDT et mirex) et les biphényles polychlorés (BPC 118, 138, 153, 170, 180 et totaux). Les chlorophénols y seront comparés.

SESSION 3A

BIOLOGICAL TESTING OF EFFLUENTS AND RECEIVING WATER TO PREDICT ENVIRONMENTAL IMPACTS/TESTS BIOLOGIQUES SUR LES EFFLUENTS ET LES EAUX RÉCEPTRICES AFIN DE PRÉDIRE LES RÉPERCUSSIONS ÉCOLOGIQUES

CHAIRPERSON/PRÉSIDENTE
Stella Swanson

BIOLOGICAL TESTING: A KEY COMPONENT OF AQUATIC ENVIRONMENTAL EFFECTS MONITORING AT PULP AND PAPER MILLS.

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BACKGROUND:

Under Section 38 of the Fisheries Act, Environment Canada has the authority to develop regulations defining "deleterious substances". The Pulp and Paper Effluent Regulations were originally promulgated in 1971. However, over the past two years, efforts have been underway to revise and update these regulations. The revised regulations have been prepared with considerable public and industry consultation and it is anticipated that they will be published in the Canada Gazette I for official comment in November, 1991. Following the 60 day public review period, the proposed regulation will be finalized and published as a new law which should be published in the Canada Gazette by early 1992. The new regulations will be quite different from the 1971 version with tighter effluent limits for biochemical oxygen demand (BOD), total suspended solids and acute lethality to fish. These regulations will apply to all pulp and paper mills in Canada. Also new to these regulations will be the requirement for all pulp and paper mills to conduct aquatic Environmental Effects Monitoring (EEM) studies on a three year cycle. Under Sections 27 to 34 of the regulation, mills will be required to submit a study design plan to the Regional Authorization Officer of Environment Canada. After approval of the study plan, the company will have two years to conduct the required EEM studies and one year to complete sample analyses, data interpretation and report the results. The EEM program will be a continuing effort with the study design for the next cycle being modified to reflect the results of the previous EEM(s) data and possible revised national program guidance. Revisions to the EEM requirements, a portion of which is described in this paper, could occur as a result of further stakeholder consultation or as a result of public input from the Gazette process.

BIOLOGICAL TESTING REQUIREMENTS

The testing and monitoring required for the pulp and paper EEM program are outlined in the Environment Canada EEM Requirement Document (EPS 1/RM/18). These requirements include mill operating information; effluent and receiving water characterization for a number of physical and chemical parameters; assessments of the benthic and fish communities in the receiving waters and sediments plus a number of biological (aquatic toxicity) tests. The EEM program is intended to integrate all of this information to determine the effectiveness of the regulation in protecting the aquatic resources in the receiving environment and to identify areas where more stringent controls may be required.

Aquatic toxicity tests have been included in the EEM program for several reasons: biological tests integrate the effects of all the chemicals found in complex pulp mill effluents; sublethal and chronic toxicity tests, in addition to the acute toxicity compliance tests required in the Regulation, are proactive measurment tools which can help in defining any zones of sublethal effect in the receiving waters; the conducting of biological tests on sediments from the receiving environment allows direct measurement of any lethal or sublethal toxic effects; and biological testing data will compliment the results of the biological monitoring and chemical data, helping to identify possible cause and effect relationships linking mill effluent discharges to environmental degradation.

There are two types of biological testing requirements: core tests which must be conducted by all mills in Canada and mandatory site-specific tests that must be conducted if certain criteria (triggers) are met at a particular mill. There are three core biological testing requirements: all effluents must be tested using a fish early life stage survival and growth test and an invertebrate reproduction test while sediments must be tested using a benthic invertebrate survival test (Table 1). Appropriate freshwater and marine/estuarine test species are recommended depending on the water body receiving the effluent discharge.

If any of the site-specific trigger criteria listed in Table 1 are met, the corresponding site-specific test must be conducted. For some of the tests cited in Table 1, there are different species options recommended with advice on appropriate species choices. Reference methods for all of the tests are cited in the EEM Requirements Document and further details regarding the study design, sampling requirements, test protocols and data interpretation are outlined in a separate EEM Technical Guidance Manual.

LEVEL OF EFFORT:

The Requirements Document sets out the minimum number of samples that must be collected and analyzed to meet the EEM legal requirements. For the core tests on effluent, six samples must be tested during the one year design phase. Characterization of effluent is also an annual requirement of the EEM program and the Regional Authorization Officer will determine the level of effort required which could range between one to six samples annually. The same minimums will apply to the site-specific mandatory tests when triggered.

For receiving water toxicity testing, a minimum of 4 areas must be sampled (control, near-field and two far-field areas). In each area, a minimum of three sampling units are required for a total of 12 samples. For the sediment tests, again 12 samples will be required. If no historical data on sediment toxicity is available, a minimum of 12 sampling areas must be sampled with one sampling unit per area. If acceptable historical data exists, then a minimum of 4 sampling areas with three sampling units per area must be tested.

QUALITY ASSURANCE/QUALITY CONTROL

The Requirement Document and Technical Guidance Manual will set out the QA/QC criteria for the toxicity testing segments of the EEM program. These criteria will include a quality assurance checklist; a quality management plan for the laboratory, a quality assurance officer on staff at the laboratory; establishment of Standard Operating Procedures; a reference toxicant testing program inplace and maintenance of a warning (control) chart system for all chemical and biological analyses offered..

FUTURE NEEDS AND ACTIVITIES

With the introduction of these new toxicity testing methods, laboratory staff in both the public and private sectors must be provided with increased training opportunities. Environment Canada must provide interpretive guidance to consultants, industry and government to help insure some measure of national consistency in data interpretation. Environment Canada is developing a national data management system to store and retrieve the vast amount of data that will be generated by the EEM program. The requirements will be reviewed and revised as required on a regular basis to ensure that the program stays current with available techniques and defined objectives. Research must continue to develop new environmentally relevant and efficient toxicity tests which will lead to the standardization of these methods for possible application in national EEM programs. With an increased demand for toxicological testing services and the development of new private sector laboratories, consideration should be given to establishing a national laboratory accreditation program independently run with resources generated from its laboratory membership to help ensure that these laboratories are producing reliable data. Continued consultation and cooperation between government (federal and provincial), consultant laboratories and the pulp and paper industry must continue in all of the above areas in order for the pulp and paper environment effects monitoring program to be successful.

TABLE: 1 BIOLOGICAL TESTING REQUIREMENTS IN THE AQUATIC ENVIRONMENTAL EFFECT MONITORING PROGRAM FOR CANADIAN PULP AND PAPER MILLS

Test Media	Core Tests	Site-Specific Tests
Effluent	- Fish Early Life Stage Development and Growth - Invertebrate Reproduction	- Plant Toxicity[1] - Genotoxicity[2] Fish Behavioural Assessment[3]
Receiving Water		- Fish Early Life Stage Development[4] - Invertebrate Reproduction[4] In-Situ Fish Lethality[5]
Sediments	- Benthic Invertebrate Survival	- Benthic Invertebrate Survival and Growth.[6]

(1) A mill using chlorine dioxide substitution or evidence of impact on plant community is demonstrated by the resource and habitat inventory assessment, (2) Statistically significant increase in fish tumours relative to reference areas, (3) Site-specific concerns or evidence of effluent-associated impact on fish migration, distribution or abundance, (4) Minimum effluent dilution in receiving water is less the mean IC_{25} of corresponding effluent tests, (5) Site-specific concerns or evidence of effluent associated impacts on fish migration, distribution or abundance, (6) No statistically significant lethal effect observed in core sediment toxicity tests.

THE ACUTE LETHALITY OF SAMPLES OF LIQUID EFFLUENT FROM PULP AND PAPER MILLS IN ONTARIO TO RAINBOW TROUT AND *Daphnia magna*.

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INTRODUCTION

As part of Ontario's Municipal-Industrial Strategy for Abatement, the mills of the pulp and paper sector were required to conduct laboratory tests (Craig et al., 1983; Poirier et al., 1988) to monitor samples of their discharges for acute lethality to trout and to *Daphnia magna*. The regulations also required chemical analyses of effluent samples for concentrations of priority pollutants. A preliminary interpretation of the toxicity results is given below concerning the possible causes of lethality. The Toxicity Test Reports for the samples (Abernethy et al., 1991a) and a summary report of the analytical chemistry data for each process effluent (MOE, 1991) were used for this interpretation.

RESULTS AND DISCUSSION

Overall, the monitoring occurred at 40 sampling locations at 27 companies for the period of January to June 1990. The Ministry received the Toxicity Test Reports for 181 trout and 208 *Daphnia* tests. For trout: 66 samples were nonlethal, 22 samples had LC50s > 100% effluent and 93 samples were lethal (LC50s < 100% effluent). For *Daphnia*: 70 samples were nonlethal, 38 samples had LC50s > 100% effluent and 100 samples were lethal.

COOLING WATER. Most samples of cooling water (33 of 37) were nonlethal to trout, 3 samples had LC50s > 100 % effluent and 1 sample was lethal. *Daphnia* toxicity tests were conducted on 42 samples: 24 were nonlethal, 12 had LC50s > 100% effluent and 6 were lethal. 5 samples from Kimberly-Clark, Terrace Bay were toxic to *Daphnia*; residual chlorine, from the municipal water supply to the mill, was the probable cause.

SULPHATE (KRAFT) MILLS. These samples were toxic and the trout test was generally more sensitive than the *Daphnia* test (except the samples from Malette, which were equally toxic to both animals). The samples from E.B. Eddy, Espanola and of Kimberly-Clark, Terrace Bay were low in toxicity to both animals. Both effluents have secondary treatment. The effluents of Boise Cascade, Fort Frances and Canadian Pacific, Dryden also receive secondary treatment but their samples still had some toxicity to trout. The most toxic samples tended to be from the facilities that did not have secondary treatment: Canadian Pacific, Thunder Bay (high resin acids and low dissolved oxygen (DO)), James River-Marathon (low pH) and Malette (low pH and high resin acids). The effluent samples from the two mills of Domtar (Cornwall and Red Rock) were toxic and resin acids were among the suspected toxicants.

SULPHITE-MECHANICAL MILLS. Most of these samples were toxic to both animals. In the *Daphnia* test, low DO was common and it could have contributed to the toxicity. It was associated with effluents of high biological oxygen demand. High concentrations of resin acids occurred frequently and could explain the usually greater sensitivity of trout compared to *Daphnia* (except the samples from St. Mary's, for which neither animal was more sensitive). The biological uptake of resin acids is highest under acidic conditions (Taylor et al., 1988) and pH itself may be toxic when it is outside the range 5.5 to 9.5. The effluent samples of low pH were from: Abitibi-Price, Thunder Bay Division, Thunder Bay, Abitibi-Price, Iroquois Falls and Boise Cascade, Kenora. The samples from Abitibi-Price, Provincial Papers and from Quebec and Ontario Paper were generally nonlethal.

CORRUGATED MILLS. No data are available on the two effluents of MacMillan Bloedel, Sturgeon Falls. The audit sample of each discharge was lethal to both test animals. Low pH, low DO and possibly resin acids were suspected causes. The effluent samples of Domtar, Trenton were toxic to both animals and resin acids and low DO were suspected causes.

BOARD-PAPER MILLS. These samples were the least toxic to both animals. The best examples were the two mills of Kimberly-Clark (Huntsville and St. Catharines). The toxic samples in this mill category were from Noranda, Thorold and from E.B. Eddy, Ottawa. The Noranda effluent is chlorinated. The E.B. Eddy samples were more toxic to *Daphnia* than to trout but the cause was unclear.

CONCLUSIONS

Overall, the data suggest that resin acids, pH extremes and low DO levels were among the primary toxicants in the samples of pulp and paper effluents. The impact of pH cannot be separated from that of the other toxicants because it can have a direct toxic action and a modifying effect on the physical, chemical and toxic properties of other effluent constituents (Abernethy et al., 1991b).

While it was common for *Daphnia* to be less sensitive than trout, there were some samples for which the reverse was true. These differences in test sensitivity were greater than the variability in the procedures themselves. For example, the effluent of E.B. Eddy, Ottawa and the January and June samples of St. Mary's were more toxic to *Daphnia*. The reason was unclear for E.B. Eddy, while low DO levels were implicated in the St. Mary's samples. The changes over time in the concentrations and mixtures of toxicants make necessary the use of both test procedures to characterize effluent toxicity. Since *Daphnia* is a filter-feeder, it may have been responding additionally to the toxicants associated with the suspended solids in these effluents.

Generalizations relating mill processes and treatment technologies to the toxicity of process or combined effluents are difficult because toxicity will be influenced by other factors such as chemical spills, overloading, differences in wood furnish and the daily operation and maintenance of "ex-plant" treatment systems.

Some of the toxicity results may reflect seasonal differences or process changes over the six month period of sampling. A more detailed analysis of all the available data for the whole year of monitoring (toxicity, chemistry and mill operations) is necessary before the cause(s) of toxicity can be confirmed with any assurance for an effluent. The interpretation of the toxicity data from the chemical characterizations was difficult because the effluent samples contained so many substances.

The toxicity tests will detect harmful concentrations and mixtures of most chemical constituents of effluents. But compliance with end-of-pipe limits for acute toxicity would not necessarily control the potential effects of environmental contamination that can be caused by the loading of bioaccumulative substances. These substances are generally nonpolar organic chemicals of high molecular weight and low water solubility. A chemical-specific assessment of this hazard is difficult because of the large number of high molecular weight substances in these effluents. However, because of their hydrophobicity, these chemicals would be mostly sorbed to the suspended solids of the effluents rather than in solution. The implication is that control of the suspended solids could, as a side benefit, also control the bioaccumulative substances without the need to identify each and every one of them.

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THE USE OF AQUATIC PLANT TOXICITY TESTS IN BIOMONITORING PROGRAMS.

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ABSTRACT

The conceptual approach of using representatives of three trophic levels to screen for aquatic toxicity has been advocated in various regulatory programs, including biomonitoring programs. In practice, however, aquatic plants have been used much less often than aquatic animals, despite evidence that aquatic plants can be the most sensitive species for a variety of pure chemicals and complex effluents. Although the U.S. EPA has included freshwater and marine algae in their manuals for chronic toxicity testing of effluents and receiving waters, few regions or states currently require these tests on a routine basis. For fresh water, a 96-hour growth inhibition test with the green alga, Selenastrum capricornutum, is specified. At present, this test has not been widely used for effluent toxicity assessment. EPA is currently developing a test procedure with the duckweed, Lemna. For marine and estuarine waters, a sexual reproduction test with the red alga, Champia parvula, has been used fairly extensively in EPA Region 1. A spore germination test with the kelp, Macrocystis pyrifera, has also been recommended for regulatory purposes. Although there are some problems (concerning experimental design and interpretation of results) associated with the use of aquatic plants to assess the toxicity of effluents and receiving waters, valid reasons exist for incorporating them into biomonitoring programs.

INTRODUCTION

Various regulatory programs and agencies mandate or recommend the use of representatives of three trophic levels for basic aquatic toxicity screening. Typically, a fish, an invertebrate, and an alga are included. Many laboratories have experience in phytotoxicity testing to support registrations of pesticides and new chemicals, under guidelines such as those developed by the OECD and the U.S. EPA (under TSCA and FIFRA). To date, however, aquatic plants have not been widely used in effluent toxicity testing programs in the United States. Although the U.S. EPA has published methods for algal toxicity testing of effluents and receiving waters, few states or regions require algal toxicity tests on a routine basis. This paper discusses the current and potential uses of aquatic plant toxicity tests in biomonitoring programs.

NEED FOR AQUATIC PLANT TOXICITY TESTS

Previous reports that aquatic plants are relatively insensitive, compared to fish and Daphnia, (Kenaga and Moolenaar, 1979; Kenaga, 1982; Morgan, 1972) led to the perception that aquatic plant toxicity data is not necessary in an aquatic hazard assessment. On the contrary, more recent studies have clearly demonstrated that aquatic plants can be just as sensitive, and in many cases, much more sensitive, than animal test species. Benenati (1990) examined the TSCA data base of Premanufacture Notices (PMN) and found that the freshwater green alga, Selenastrum capricornutum, was more sensitive than fish and daphnia in over half of the PMNs that contained phytotoxicity data. Aquatic plants have also been observed to be more sensitive than animal species to a variety of metals, insecticides, herbicides, pulp mill and industrial effluents, soil elutriates and hazardous waste leachates (Taraldsen and Norberg-King, 1990; Walsh et al., 1982; Schimmel et al., 1989a; Schimmel et al., 1989b; Thomas et al., 1986). Of course, plant and animal test systems are fundamentally different and the means of expressing the results are not always comparable, rendering the debate about relative sensitivity rather fruitless. However, it is intrinsically logical that we should not expect animal toxicity data to adequately serve as a surrogate for the entire plant kingdom. This insight, coupled with the ecological relevance of algae and aquatic macrophytes as the basis of the aquatic food chain, points to the need for aquatic plant toxicity data in any aquatic hazard assessment.

CURRENT TESTING SCENARIO: FRESHWATER

In the mid 1960's an algal test method was developed and evaluated by representatives of industry, academia and government. This method, first published in 1971 as the "Algal Assay Bottle Test" (U.S. EPA, 1971) and later updated (Miller et al., 1982), was not specifically designed as a toxicity test but rather as a test to determine the limiting nutrients and growth potential of natural waters. However, it is this method, with minor modification, that serves as the basis for most current toxicity testing protocols.

The U.S. EPA manual for chronic toxicity testing with freshwater organisms (U.S. EPA, 1989) describes a 96-hour growth inhibition test with the unicellular green alga, *Selenastrum capricornutum*. In this method, replicate flasks containing various concentrations of effluent diluted with either culture medium or receiving water are inoculated with algae. (The effluent must first be filtered using a 0.45 micron porosity filter. Also, nutrients are added to the effluent prior to preparing the test dilutions to ensure that all treatments contain enough nutrients to support growth). At the end of 96 hours, algal growth in each flask is measured by either cell counts, chlorophyll content or turbidity. Population growth in the treated cultures is compared to that in the control. The endpoints may be expressed based upon hypothesis testing (LOEC and NOEC) or point estimation (IC50). The most significant flaw in this test method is the mandated exclusion of the chelating agent EDTA from the medium, apparently out of concern that the presence of EDTA would mask metal toxicity. Available data, however, (Greene, 1988) indicate that a minimal concentration of EDTA must be present for optimal growth and good agreement between replicate flasks.

There appears to be little experience with the freshwater algal chronic test. In the EPA's "Program Survey - Biological Toxicity Testing in the NPDES Permits Program" (U.S. EPA, 1987), only three states (Illinois, Oregon and Washington) mention using the *Selenastrum capricornutum* test. The Illinois Environmental Protection Agency laboratory conducted algal toxicity tests on a number of effluents, following the U.S. EPA procedure with the exception that EDTA was included. Tests were felt to be difficult to conduct and interpret, as stimulation was observed as often as inhibition (R. Mosher, personal communication). Currently, in the permitting process in these three states, algal toxicity testing is required only on selected (generally industrial) effluents.

CURRENT TESTING SCENARIO: MARINE

The U.S. EPA manual for chronic toxicity testing with marine and estuarine organisms (U.S. EPA, 1988) describes a sexual reproduction test with the marine macroalga, *Champia parvula*. In this method, male and female gametophytes are exposed to various concentrations of effluent for two days, followed by a 5- to 7-day period of development in control medium. Each test solution must contain a minimum of 50% natural seawater; thus, the maximum concentration of effluent that can be tested is 50%. The response is measured as the number of cystocarps (evidence of sexual reproduction) per female. Data from this test are expressed in a manner more similar to the chronic toxicity tests with animals than are data from tests with microalgae, where population growth is the measured response. The endpoints for the Champia test are typically reported as the NOEC and LOEC.

The Champia test has been required on a routine basis in the NPDES permitting process in EPA Region 1. Available data indicate that this test is quite sensitive and questions have been raised concerning the interpretation of the data from this test and the applicability of this species as a surrogate for all seaweeds (G. Thursby, personal communication).

ADVANTAGES AND DISADVANTAGES OF CURRENT TESTS

Aquatic plants are advantageous to use as toxicity test organisms because they are comparatively easier to maintain in laboratory cultures than are fish and invertebrates. Aquatic plant cultures do not require daily feeding or cleaning, merely a regular (usually weekly) transfer into fresh growth medium. Aquatic plant toxicity tests can be conducted in relatively small volumes (25 - 100 mL per test vessel).

A problem with the use of microalgae is that the test design must be static, and it is often desirable to test effluents under flow-through or renewal conditions. It is conceivable that a continuous culture method could be devised, but it would probably be impractical for routine biomonitoring. Also, filtering the effluent, which is necessary in microalgal tests, can alter toxicity. New test methods for the duckweed, discussed below, avoid these problems.

Interpretation of aquatic plant toxicity data is sometimes complicated by stimulatory responses. The biological significance of stimulation has yet to be adequately addressed by the scientific community. In addition, more discussion is needed on the selection of the appropriate endpoints and the correct statistical methods for calculating endpoints from aquatic plant toxicity tests (Hughes et al. 1988).

FUTURE TRENDS

There is considerable interest in the development of a freshwater effluent toxicity test method with an aquatic macrophyte, such as duckweed. Duckweeds are found floating on the water surface and serve as food and shelter for other organisms. Duckweed can be tested under static, static renewal or flow-through conditions. (Wang, 1986; Walbridge, 1977; Davis, 1981; Taraldsen and Norberg-King, 1990). In addition, the effluent samples do not need to be filtered for tests with macrophytes. The species that have garnered the most attention as potential test organisms are Lemna gibba and Lemna minor. A "Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3" has just been published (ASTM, 1991), although it does not specifically address effluents. A test method with Lemna minor is expected to be published in the 17th edition of Standard Methods for the Examination of Water and Wastes (Wang, 1990), and work at the EPA's National Effluent Toxicity Assessment Center (NETAC) has also led to a method for Lemna minor (Taraldsen and Norberg-King, 1990). The test medium (dilution water) is a defined nutrient solution in the former two protocols, while the NETAC method favors the use of soil to prepare the medium. Test duration ranges from 4 to 7 days in these methods, and the response is typically measured by the increase in frond number, although other parameters, such as chlorophyll or dry biomass, may be used. As with microalgae, population growth is measured, and the endpoints may be reported as NOEC and LOEC or IC values.

Other freshwater macrophytes may also be suitable for effluent toxicity testing. Wang (1986b; 1987) has used Japanese millet (Echinochloa crugsalli) in toxicity testing of single compounds and complex mixtures.

For marine and estuarine waters, various species of microalgae could be incorporated into biomonitoring programs. The chain-forming marine diatom, Skeletonema costatum, has been used in other regulatory programs (TSCA, FIFRA, dredged material disposal assessment), while a more rapidly growing diatom, Minutocellus polymorphus, has been recommended because it occurs as single cells (Walsh et al. 1988). Marine macrophytes other than Champia parvula may also be useful. A 48-hour spore germination test with the giant kelp, Macrocystis pyrifera, (Anderson et al., 1990) has been recommended for regulatory purposes in the State of California.

CONCLUSIONS

1. By virtue of their importance in nutrient cycling, oxygen production, and as the basis of the food chain, aquatic plants should be included in biomonitoring programs. Data from animal species cannot be expected to be protective of plants, and available data indicates that they are not.

2. While both freshwater and marine microalgae can be used in biomonitoring programs, this has not been the case to date. Because effluents must be filtered and only static non-renewal exposures are practical for microalgae, attention has been shifted to methods for macrophytes. Alternative microalgal species and response parameters (such as ATP) that would shorten test duration could enhance the utility of microalgae.

3. Effluent toxicity test methods with the marine macrophyte, Champia parvula, have been well developed and extensively used. This species appears to be extremely sensitive, showing a response with almost all effluents. For freshwater, a macrophyte such as Lemna minor holds promise as a test organism. Other macrophytes may also be useful.

4. The biological significance of various endpoints should be considered, as well as the validity of the statistical procedures used in their determination.

5. In developing aquatic plant toxicity tests for biomonitoring programs, it is important that test methods be validated on a variety of effluents in a number of laboratories prior to the imposition of testing requirements upon permittees. Desirable traits of aquatic plant toxicity test protocols include: the use of an ecologically important species that is easy to culture; the use of a defined growth medium and optimal testing conditions; a short test duration to minimize effluent changes; and a sensitive, yet well-defined and relevant test endpoint.

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EVALUATION OF THE TOXICOLOGICAL EFFECTS OF OIL DISPERSANTS BY MODELED-EXPOSURE TOXICITY TESTING.

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ABSTRACT

By virtue of their nature and usage, the exposure potential of aquatic organisms to oil dispersants is highly ephemeral. To address this circumstance, in addition to traditional constant-concentration exposures, more realistic spiked-exposure, continuous-flow toxicity tests using the oil dispersant Corexit 9527® were performed using the early life stages of four California marine species. Test chambers containing sensitive life stages of the giant kelp *(Macrocystis pyrifera)*, the red abalone *(Haliotis rufescens)*, a kelp forest mysid *(Holmesimysis costata)*, and the topsmelt *(Atherinops affinis)*, were inoculated with concentrated dispersant, then allowed to flush with clean, filtered seawater. Spectrophotometric monitoring of tests showed dispersant levels diminishing to below detection limits within 5 to 6 h or less. Comparison of spiked-exposure results with previous data obtained using the same species and dispersant under constant-exposure conditions showed higher values for both EC/LC50s and NOECs under spiked conditions. Results showed *Haliotis* to be the most sensitive species tested, with *Atherinops* being least sensitive in terms of NOEC and *Holmesimysis* being least sensitive in terms of median effect concentration; *Macrocystis* was intermediate in both measures. When spiked- and constant-exposure toxicity test results were compared, no consistent conversion factor which might relate the two datasets was found.

INTRODUCTION

Under existing standard use guidelines, chemical dispersants may only be applied to oil spills under certain environmental conditions; sea swell, wind and wave intensity must all be sufficiently high to achieve proper chemical mixing (Raj and Griffith, 1979; NRC 1989). Under these conditions, the likelihood of dispersant concentrations remaining constant in the water column is extremely small. In fact, most guidelines are specifically fashioned to avoid such a condition.

Traditionally, acute dispersant toxicity has been described using constant toxicant concentration exposures (classical 24- to 96-h toxicity tests [NRC, 1989; Singer *et al.*, 1990a]). While they may allow for comparison between different studies and dispersant types, they provide a poor estimate of the toxic effects which may occur in actual use. Recent studies have reported initial dispersant concentrations during sea trials of <1 up to 13 ppm at various depths and times after application (Canaveri and Lindblom, 1976; Bocard *et al.*, 1984; Griffiths *et al.*, 1981; Mackay and Wells, 1983) . However, in all cases concentrations dropped below detectable limits within a matter of hours.

It has previously been demonstrated with pesticides that short-term episodic exposures (typically ≤6 h) can elicit delayed toxic effects not seen in constant-exposure tests (Heming *et al.*, 1988; Heming *et al.*, 1989). In such cases, traditional constant-exposure test data may underestimate a toxicant's effects. Conversely, with some toxicants short, burst exposures may allow for greater amelioration of toxic effects by detoxication or other "repair" processes.

The toxic effects of the dispersant Corexit 9527® on the early life stages of four California marine species were initially studied using traditional constant-concentration exposure regimes in order to produce a dataset which might be compared to past studies and to give a baseline "worst case" toxicity estimate (Singer *et al.*, 1990a). Subsequent to that, another study was undertaken to investigate the toxic effects of spiked exposures of the oil dispersant Corexit 9527® on these same species. Spiked exposures, as opposed to pulsed or other episodic forms of exposure, refers to the method of dosing by which a specific quantity of toxicant is added to the test chamber all at once, followed by an immediate initiation of dilution by direct flow of diluent through the test chamber.

The four species tested: a canopy forming Laminarian alga, the giant kelp *(Macrocystis pyrifera)*; a shallow subtidal gastropod, the red abalone *(Haliotis rufescens)*; a common kelp forest mysid *(Holmesimysis costata)*; and an important near-shore Atheriniform fish, the topsmelt *(Atherinops affinis)*, represent a wide diversity of trophic and taxonomic levels. A spiked-exposure regime was used here in an attempt to more accurately model exposures which might occur in actual use situations. The spiked-exposure data were meant to provide more applicable toxicity estimates for use by oil spill on-scene coordinators, and also hopefully provide a framework for relating existing constant-exposure toxicity data to more realistic situations.

MATERIALS AND METHODS

Exposure system

Both constant- and spiked-exposure tests with Corexit 9527® (Exxon Chemical Corp., Houston, TX) were carried out under closed, flow-through conditions; specific components of the system have been previously described (Singer *et al.*, 1990b). Constant exposure testing involved premixed test solutions of the dispersant which were made daily and pumped through the exposure chabers (Singer *et al.*, 1990a). In spiked-exposure tests organisms were loaded into exposure chambers prior to testing. Initial exposure concentrations were achieved by spiking chambers with decreasing amounts of a 1,000 ppm (1 ppt) v/v Corexit 9527® stock solution and then flushed with aerated, filtered seawater (Singer *et al.*, 1991).

Test organisms

Macrocystis zoospores were obtained from sporophyll collected by divers from inshore kelp beds south of Carmel, California, U.S.A. Swimming zoospores for use in toxicity tests were released upon rehydration of sporophyll after overnight desiccation (Anderson and Hunt, 1988). Fertilized *Haliotis* embryos were obtained from spawning of laboratory brood stock animals using established methods (Ebert and Houk, 1984). Juvenile *Holmesimysis* were released in the laboratory from wild gravid females collected off Monterey, California, U.S.A. After partutition, mysid juveniles were reared for 4 d on newly hatched *Artemia* nauplii (Martin *et al.*, 1989). *Atherinops* larvae were hatched from laboratory collected eggs using previously developed techniques and were reared for 10 d on newly hatched *Artemia* nauplii (Anderson *et al.*, 1990; Middaugh *et al.*, 1990).

Test procedures

Short-term toxicity tests were employed in both constant- and spiked-exposure testing; kelp and abalone tests lasted 48 h and involved non-lethal endpoints (gametophyte growth and developmental abnormality, respectively), while mysid and topsmelt tests were 96 h in duration and measured lethality. Water temperature, dissolved oxygen and pH were monitored daily during testing. Natural seawater filtered to 1 µm (sand, cotton cartridge and activated charcoal filters) was used in all tests at ambient salinity (typically 34 ‰).

Table 1. Comparison of mean (SD) NOEC and LC50 values for Corexit 9527® under spiked- and constant-concentration exposure regimes. Constant exposure data taken from Singer *et al.* 1990a, 1991. Data are mean values for replicate tests (n = 3).

	Spiked Exposure		Constant exposure	
Species	NOEC	LC50[a]	NOEC	LC50[a]
Haliotis	6.6 (1.6)	15.9 (2.3)	1.1 (0.4)	1.6 (0.3)
Holmesimysis	14.6 (6.1)	140.1 (21.7)	3.3 (1.4)	6.2 (1.7)
Atherinops	57.0 ()	83.0 (22.3)	13.5 (1.0)	30.7 ((8.1)
Macrocystis	≈14 (2.1)[b]	92.6 (8.3)	≈1.8 (0.5)[b]	NC

NC = not calculated.

[a] EC50 for *Haliotis*; IC50 for *Macrocystis*.

[b] Approximate values; one NOEC value was below lowest test concentration.

Testing involved six treatments: five dispersant concentrations and a clean dilution water control. In constant-exposure tests, the premixed test solutions were pumped through the chambers at a constant rate of 2 mL min^{-1}. In spiked-exposure tests, immediately upon completion of dispersant addition (or organism addition in the case of abalone) flushing of exposure chambers with clean seawater was initiated at a rate of 2 mL min^{-1}. Dispersant concentrations were monitored during both studies by UV spectrophotometry with a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer (detection limit 0.3 ppm [Singer *et al.*, 1990b]).

The same general test procedures were used in both exposure regimes (Singer *et al.*, 1990a, c). *Macrocystis* germ tube lengths were examined at 400x under a light microscope and measured with an ocular micrometer after 48 h. *Haliotis* tests also lasted 48 h; after after that time veliger larvae were examined microscopically for normal or abnormal shell developement. Four-day-old juvenile *Holmesimysis* and ten-day-old larval *Atherinops* were observed for mortality for 96 h after spiking. Both species were fed newly hatched *Artemia* daily during testing.

RESULTS

In constant-exposure testing no effect concentrations for the four species ranged from 0.63 ppm in abalone to 13.9 ppm in topsmelt, while median effect concentrations ranged from 1.6 to 40.6 ppm (Singer *et al.*, 1990a). *Haliotis* was seen to be the most sensitive species in terms of both NOEC and EC50, with mean values of 1.1 and 1.6 ppm Corexit, respectively (Table 1). *Macrocystis* was next in sensitivity, with a mean NOEC of approximately 1.8 ppm. Juvenile *Holmesimysis* were several-fold less sensitive than were abalone larvae, with a mean NOEC of 3.3 ppm and mean LC50 of 6.2 ppm. Finally, larval *Atherinops* showed a mean NOEC of 13.5 ppm with a mean LC50 of 30.7 ppm (Table 1). Reproducibility of results from replicate tests of each species was high (Singer *et al.*, 1990a).

Results from spiked-exposure testing generally showed a smaller overall difference between the most and least sensitive species (Singer *et al.*,1991). Also, spiked-exposure tests resulted in lower overall sensitivities across all species (Table 1). *Macrocystis* gametophyte germ tube length NOECs ranged from 12.2 to 16.4 initial ppm in replicate tests , while IC50 values averaged 92.6 initial ppm. *Haliotis* was the most sensitive species tested, with NOEC and EC50 values 6.6 and 15.9 initial ppm, respectively. *Holmesimysis* was intermediate in sensitivity in terms of NOEC but least sensitive in terms of median effect concentration (Table 1). NOECs for *Holmesimysis* tests were somewhat variable, ranging from 8.4 to 20.5 initial ppm; LC50s were more

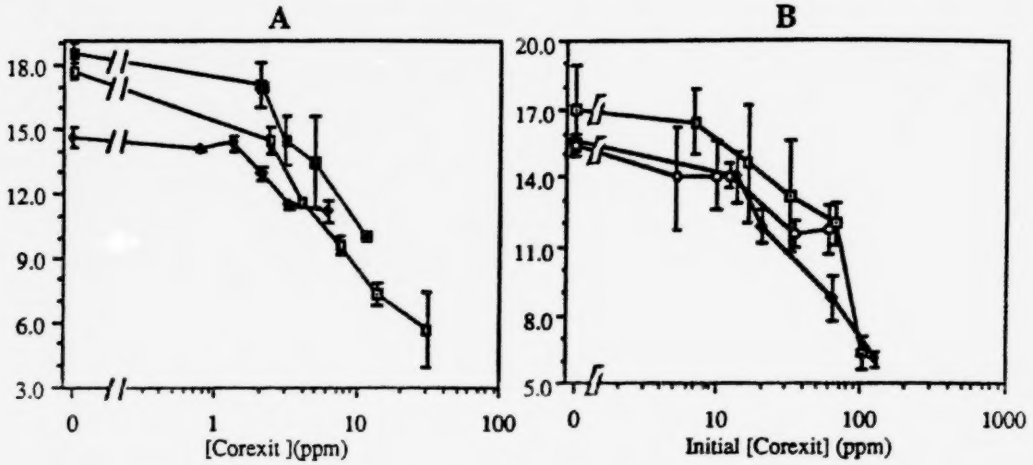

Figure 1. Macrocystis gametophyte germ tube lengths in triplicate toxicity tests under A) constant- and B) spiked-concentration exposure regimes.

consistent, ranging from 120.4 to 163.4 initial ppm. *Atherinops* was the least sensitive and most variable species in terms of NOECs, which had a mean of 57.0 initial ppm . LC50s for the replicate tests averaged 83.0 initial ppm (Singer *et al.*, 1991).

DISCUSSION

Concentrations of the dispersant Corexit 9527® which were found to be toxic under spiked-exposure conditions were predictably higher than those for constant toxicant exposures; however, spiked-exposure test results were somewhat less variable across species. NOECs for the four species tested differed by about 10-fold between most and least sensitive under spiked conditions, while constant-exposure tests have yielded results which varied across species by about 13-fold (Table 1). Median effect concentrations varied between most and least sensitive species by approximately 10-fold in spiked-exposure tests as opposed to nearly 15-fold under constant-exposure conditions. Differences in spiked- versus constant-exposure NOECs were more consistent than were LC50 values. Mean spiked-exposure NOEC values were 4 to 7 times higher than constant-exposure NOECs for all four species (Table 1). Differences in mean LC50 values were much less consistent, ranging from about 3 to over 20-fold higher in spiked-exposure tests. No distinct pattern was evident across either taxonomic or developmental levels; *Atherinops* had the most similar mean LC50 values (83 ppm for spiked-exposure tests versus 30.7 ppm for constant), *Holmesimysis* had the least similar values (140.1 ppm for spiked-exposure tests versus 6.2 ppm for constant), while *Haliotis* was intermediate (15.9 ppm for spiked-exposure tests versus 1.6 ppm for constant).

Direct comparison of spiked- and constant-exposure test results is difficult; even though both concentration and duration of exposure may not contribute to toxicity equally, it is intuitive that both have an effect. It is not surprising that a short, episodic exposure to a toxicant should require higher concentrations to elicit a toxic response than constant-concentration exposures. However it is important to note the difference in response within and across species when exposed to spiked- versus constant-concentrations. *Macrocystis* and *Haliotis*, the two developmental tests, displayed similar dose-response relationships in

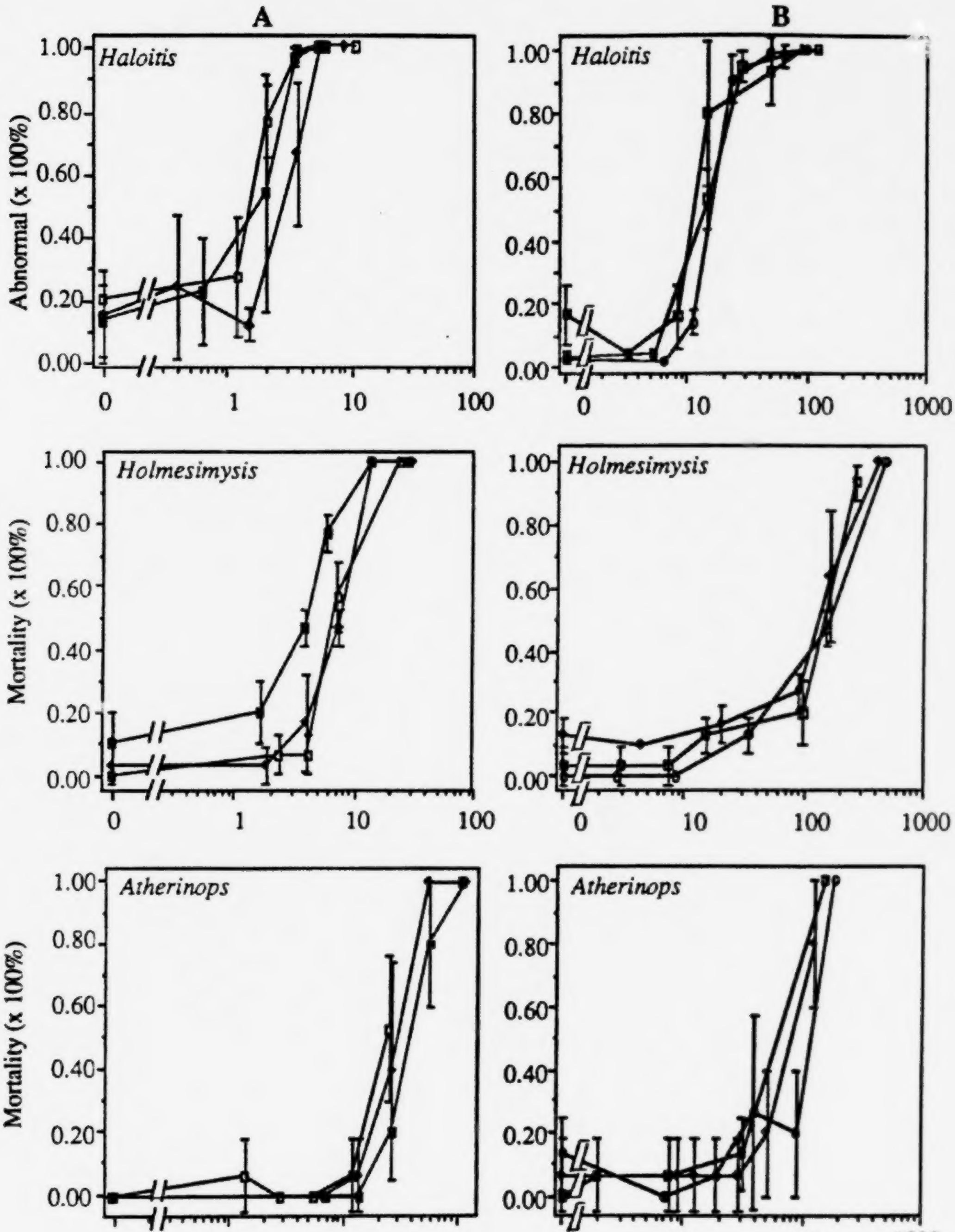

Figure 2. Dose response curves for triplicate toxicity tests under A) constant- and B) spiked-exposure testing regimes.

the two exposure regimes (Fig. 1, 2). Kelp gametophyte length decreased relatively linearly with increased initial dispersant concentration under both constant- and spiked-exposure conditions. Abalone larvae also had similar dose-response relationships in the two different exposure regimes. During test endpoint evaluation individuals of both species, after exposure to sufficiently high concentrations, were seen to have their outer cell structures disrupted and the material inside lysed into disassociated masses of cells or cell fragments.

The ability of surfactants to alter cell osmotic permeability has been discussed in both plants and animals (Pybus, 1973; Abel, 1974). The similarity in toxic effect on kelp and abalone implies a similar mode of action. In both species, test organisms are in early developemental stages and are experiencing rapid cell growth and, in the case of abalone, multiplication. At this early stage, individuals of both species are highly susceptible to cellular aberrations; any disruption or malfuction of osmotic or electrolytic activity will result in significant stress. At such an early stage individuals would not have sufficiently developed protective mechanisms. Disruption of electrolytic balance has been suggested as the mode of germ tube growth inhibition by copper in *Macrocystis* gametophytes (Anderson and Hunt, 1988). Disruption of gametophyte cell walls by the surfactants in Corexit 9527® may be responsible for the observed toxic effects. It has been previously suggested that anionic surfactants may have a chelating effect on calcium, a primary inorganic component of mollusc shells (Tarazona and Nunez, 1987). Thus, calcium robbing may be at least partly responsible for shell abnormalities seen in *Haliotis* larvae which survive the initial physiological stress of intermediate dispersant exposures.

Mysid data showed a fairly steady decline in survival throughout the test durtion in both exposure regimes (Singer *et al.*, 1990a, 1991). Spiked-exposure results indicated a significant amount of delayed mortality up to 96 h after dispersant exposure.

Unlike mysid juveniles, *Atherinops* larvae in both exposure regimes displayed a measure of resistance to the toxic effect of the dispersant. In both sets of tests the majority of the mortality observed occurred in the first 24 h of the test; daily LC50 estimates showed only a 20 to 30% decline over the 4 d duration in constant-exposure tests and declined less than 10% from the first to fourth day in spiked-exposure tests (Singer *et al.*, 1990a, 1991). The data obtained suggest that in relatively intense, episodic exposures the trend of lower sensitivity with later developmental stage or higher taxanomic level (Wells, 1984) may not hold true.

Dispersants have generally been found in only the upper 5 to 10 m of the water column during sea trials (Canevari and Lindblom, 1976; Griffiths *et al.*, 1981; Mackay and Wells, 1983). Of the four species tested, mysids are likely to be the most susceptible to dispersant contact in the field by virtue of the presence of newly released juveniles in nearshore kelp canopies during most of the year. Topsmelt larvae may also be vulnerable to dispersants during spring and summer. As in many fishes, these larvae occur within the upper few meters of the water column where contact with dispersants would be most probable (Allen, 1982). Abalone embryos would be mainly susceptible following dispersant application along rocky shores and in shallow subtidal areas. Newly germinated kelp spores occur on the substrate in nearshore subtidal waters to depths of 30 m or more and would be the least susceptible of the four species. Thus, dispersant use in nearshore waters may pose a serious risk to some of these species.

Results of this work demonstrate that inferring toxic effects of dispersants in actual use situations from constant-exposure test data may lead to erroneous conclusions regarding environmental impacts. Traditional constant-exposure data obtained for a particular species may not give adequate insight into delayed mortality or increased or decreased sensitivity under realistic exposures. Also, differences in median effect values between spiked- and constant-exposure tests were not consistent across species; thus, no general rule can be established which would provide for the use of past toxicity data to predict toxic effects of dispersants on field populations. However, while spiked-exposure tests remain imperfect models of the "real world", our results demonstrate that even very ephemeral exposure to dispersants at field-related concentrations may be toxic to some marine larvae. Therefore, on-scene coordinators must exercise caution when considering dispersant use.

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DIAGNOSIS OF EFFLUENT ECOTOXICITY WITH AN INNOVATIVE BIOLOGICAL/CHEMICAL APPROACH. G. Costan, Environnement Canada, Centre Saint-Laurent, Montréal, Québec.

To properly assess the hazards of complex effluents, an integrated biological/chemical approach must be used. A detailed appraisal on the ecotoxicity of a specific industrial effluent will be presented. The following points were investigated: 1) organic and inorganic chemical characterization of the effluent, 2) lethal, sublethal, and chronic effects on two fish (Salmo gairdneri and Pimephales promelas) and one crustacean (Ceriodaphnia reticulata) species 3) sublethal and chronic effects on algae (Selenastrum capricornutum) and bacteria (Photobacterium phosphorem and Escherichia coli). Chemical parameters were quantified and related to observed effects. The results indicated lethal effects on the two fish and the crustacean species (>10 Toxic Units). Sublethal effects were found on: 1) ATP energy metabolism of S. gairdneri (13 - 18 T.U.), 2) growth weight inhibition of P. promelas (25 T.U.), 3) growth inhibition of S. capricornutum population (17 T.U.), and 4) on light emission of P. phosphoreum (3.8 T.U.). Chronic toxicity was found on C. reticulata reproduction (588 T.U.). The effluent was also genotoxic as indicated by a significant dose-response obtained with the SOS Chromotest. Ammonia, suspended solids, zinc, cyanides, and some organic compounds were identified as the major causes of effluent ecotoxicity.

COMPARISON OF CHEMICALS CHARACTERISTICS OF EFFLUENTS FROM 3 CANADIAN KRAFT PULP MILLS. J.H. Carey, D.T. Bennie and B.K. Burnison, Environment Canada, Rivers Research Branch, National Water Research Institute, Burlington, Ontario.

Pulp mills have been identified as significant sources of organic contaminants. Most characterization studies in the past have focused on bleach plant effluent, not whole mill effluent. Due to chemical reactions after mixing, the analytical characteristics of separate bleachery effluent streams may not be a good indication of that which is being discharged. A sampling program was conducted at the Repap Manitoba mill in The Pas, MB, which is an unbleached kraft mill with secondary treatment, the CPFP mill at La Tuque, PQ, which is an old bleached kraft facility using molecular chlorine for bleaching and primary treatment, and the E.B. Eddy mill at Espanola, ON, a modern bleached kraft operation with oxygen delignification, chlorine dioxide substitution and secondary treatment. The major organic compounds in the final effluents from all 3 mills were characterized by GC/FID, GC/ECD and molecular weight distribution. The results should be useful in identifying components which will be candidates for toxicity testing.

DEVELOPPEMENT D'UN TEST DE LÉTALITE ALGALE PAR CYTOMÉTRIE EN FLUX.

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La détermination de la toxicité létale chez les algues constitue une étape importante pour l'évaluation écotoxicologique des xénobiotiques. L'absence de bioessai sur la létalité algale nous a amené à développer ce test chez l'algue verte Selenastrum capricornutum. La cytométrie en flux associée à l'emploi d'un fluorochrome supravital (diacétate de fluorescéine: FDA) fut utilisée pour estimer les effets algicidaux. Suite à des expérimentations à l'aide de toxiques de référence (sulfate de cuivre et chlorure de mercure), il est apparu important d'effectuer certains contrôles (i.e. emploi d'un deuxiéme indicateur de viabilité, prise de lectures avant et aprés l'ajout du FDA, validation du fenêtrage cytométrique des cellules vaibles et mortes). Des CL$_{50}$ ont été déterminées pour le Cu^{2+} (42.19 ppb) et le Hg^{2+} (22.52 ppb). Des CL$_{50}$ ont également été établies pour plusieurs herbicides. Les métolachlore sur l'algue verte seront aussi discutés.

INFLUENCE DE L'EAU DE DILUTION SUR LES RESULTATS DE BIOESSAIS EFFECTUES SUR DES ENCHANTILLONS D'EFFLUENTS INDUSTRIELS.

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Une étude comparative fut menée afin d'evaluer l'influence d'eaux de dilution sur les résultats de bioessais effectués en laboratoire avec P. phosphoreum (Micortox®) et S. capricornutum exposés à divers échantillons d'effluents industriels. L'eau d'entrée de l'usine (i.e. provenant en amount dur cours d'eau récepteur), ajustée à 2% NaCl pour le biotest Microtox, a constitué l'une des solutions de dilution pour les deux biotests. Le diluant Mircotox et l'eau Millipore SQ composérent l'autre solution de dilution pour les biotests bactérien et algal, respectivement. Dans l'ensemble, l'eau d'entrée, en tant que diluant de l'echantillon, a engendré de plus faibles toxicités. Ainsi 87% et 74%, selon le bioessai algal et bactérien respectivement, des 23 échantillons analysés présentaient une toxicité ,oimdre avec l'eau d'entrée qu'avec l'eau SQ ou le diluant Microtox. Cette constatation suggére que les diluants artificiels peuvent sur vévaluer la toxicité des effluents industriels et mésestimer l'impact environnemental subséquent, et que les eaux réceptrices, dans la mesure du possible, devraient être utilisées préférentiellement comme agents de dilution lors la réalisation de bioessais en laboratoire.

INTEGRATION OF PHYSICAL, CHEMICAL, AND BIOLOGICAL DISCHARGE EFFECTS IN RECEIVING ENVIRONMENTS.

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Case studies of pulp and paper mill discharges to river, lakes and estuaries will be used to illustrate the principles of Environmental Effects Monitoring. Plume discharge models will be related to surface water quality guideline zones of non-compliance, acute lethality zones, sublethal effect zones and local receiving water uses. Fisheries and benthic study data will be related to projected plume effects to provide interpretation of long term ecological responses and identifying measurable improvements in the environment resulting from mill remedial programs.

TOWARDS A MORE COMPREHENSIVE USE OF BIOANALYTICAL TOOLS FOR INDUSTRIAL EFFLUENT CONTROL.

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Effective control of industrial effluents imposes paradoxical needs. While on one hand it is imperative to use only standardized and well established analytical methodologies, it is also essential to continuously remaim at the "state of the art" and use the most revealing and cost-efficient methodologies available. Outcome of the lengthy negotiations needed between effluent regulators and industrial polluters can often result in the use of outdated and costly standardized methodologies. An approach must be established permitting continuous updating of appropriate methodologies and new environmental concerns. A strategy to solve this dilemma, using bioanalytical tools in a more comprehensive manner is presently being suggested, through the Canadian federal ST. LAWRENCE RIVER ACTION PLAN. Quantifiable control level would be based on LOEC and NOEC responses, where a scale integrating multiple effects data from different trophic levels would determine effluent biomonitoring needs.

SESSION 3B

ENVIRONMENTAL EFFECTS MONITORING USING INDIGENOUS BIOTA IN RECEIVING WATERS/SURVEILLANCE DES RÉPERCUSSIONS ENVIRONMENTALES À L'AIDE DE BIOTE INDIGÈNE DANS LES EAUX RÉCEPTRICES

CHAIRPERSON/PRÉSIDENT
Peter Hodson

TENNESSEE'S EAST FORK POPLAR CREEK: A BIOLOGICAL MONITORING AND ABATEMENT PROGRAM.

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In May, 1985, a Biological Monitoring Program was developed for East Fork Poplar Creek (EFPC) in eastern Tennessee, United States. This stream originates within the Oak Ridge Y-12 Plant that produces nuclear weapons components for the Department of Energy. Water and sediment in the stream contain metals, organic chemicals, and radionuclides from releases that have occurred over the past 45 years. Effluents discharged from the Y-12 Plant enter the stream near its headwaters; further downstream the creek also receives urban and some agricultural runoff and effluent from the City of Oak Ridge's Wastewater Treatment Facility (WTF). Classified uses of EFPC, as designated by the Tennessee Department of Environment and Conservation, include growth and propagation of fish and aquatic life, and recreation, including fishing and swimming. Primarily because of elevated concentrations of mercury, fishing and swimming in EFPC have been prohibited since November, 1982.

The biological monitoring program was developed under mandate of the National Pollutant Discharge Elimination System (NPDES) permit as an alternative approach to compliance with water quality standards. The monitoring program has two major objectives: first, to determine if the effluent limitations established for the Y-12 Plant, as stipulated in the NPDES permit, protect and maintain the classified uses of the stream; and second, to document environmental improvements from the implementation of a water pollution control program at the Y-12 Plant. This program seeks to eliminate direct discharges of wastewaters to EFPC and to reduce inadvertent release of pollutants.

The biological monitoring program includes four major tasks: (1) ambient toxicity testing; (2) bioaccumulation studies; (3) biological indicator studies; and (4) ecological monitoring of stream communities, including periphyton, benthic macroinvertebrates, and fish. Biological conditions are monitored at six sites on EFPC ranging from kilometer 24.4 near the headwaters to kilometer 6.3 near the mouth. A site on Brushy Fork, a stream just north of Oak Ridge, is used as a reference.

Ambient (instream) toxicity was monitored through the use of 7-day static-renewal tests that measured the survival and growth of fathead minnow (*Pimephales promelas*) larvae and the survival and reproduction of a microcrustacean (*Ceriodaphnia dubia*). Full-strength water from EFPC within the Y-12 Plant boundary was frequently toxic to *Ceriodaphnia*, but less frequently toxic to the minnow larvae. Chlorine has been identified as an important toxicant in upper EFPC.

Water samples from six sites in EFPC downstream from the Y-12 Plant boundary were tested eight times with both species during a 2-year period (October, 1986 through October, 1988). These sites were ranked by the number of times they were "best" or "worst" for each species. The results of the ranking procedure revealed no longitudinal pattern to water quality in the creek based on either fathead minnow growth or *Ceriodaphnia* fecundity in 7-day tests.

Water samples collected for use in the ambient toxicity tests were routinely analyzed for conductivity, pH, alkalinity, hardness, total residual and free chlorine, and temperature. Water temperature was recorded at each site as the sample was collected; all other measurements were made in the laboratory within 3 h after sample collection. The influence of effluent from the Oak

Ridge WTF on chemical conditions in EFPC was easily detected based on measurements of conductivity, alkalinity, and total residual chlorine. The sum of the Pearson correlations between ranked values for conductivity, alkalinity, and hardness, subtracted from 3.0 provided a useful measure of the degree to which stream sites were chemically perturbed. For pristine streams on the Oak Ridge Reservation this index ranges from 0.2 to 0.4 (i.e., high correlations occurred consistently between conductivity, alkalinity, and hardness). The index's values for EFPC sites were not less than 1.5 and exceeded 2.0 within the Y-12 Plant boundary and at the site just downstream from the Oak Ridge WTF.

Both the Y-12 Plant and the Oak Ridge WTF release nutrients that enhance algal growth in EFPC. Periphyton community studies indicated that physical and/or biotic factors may contribute to the differences in algal biomass, production, and condition among the EFPC study sites. In upper reaches of EFPC, periphyton are strongly influenced by chlorine, while periphyton just below the Y-12 Plant accumulate metals (such as Hg and Cd) that may be transferred to higher trophic levels by grazers.

Bioaccumulation studies indicate that fish from EFPC have elevated concentrations of mercury compared to fish from a reference stream (30% of the EFPC fish exceeded the FDA tolerance limit of 1 μg/g). Mean concentrations of mercury in redbreast sunfish were highest near the Y-12 Plant and decreased steadily with distance downstream. Linear regression analysis of mercury concentrations in redbreast sunfish vs. time indicate that mercury contamination in this species has increased a small but statistically significant amount since monitoring began in May 1985 (average increases at the various sampling sites were from 0.2 to 0.8 μg/g). A pattern similar to that described for mercury also was evident for PCBs. From studies using caged Asiatic clams, the upper reach of EFPC has been identified as an important source of PCBs. The clam studies also indicated the presence of low levels of PAHs in EFPC.

Bioindicators of fish health revealed a downstream gradient of increasing fish health. Fish from the lower reaches of EFPC showed improvement in health over the study period but the health of fish from the upper reaches has not improved. Bioindicator responses indicate that decreased health of fish from EFPC was due primarily to toxicant exposure. Higher concentrations of detoxification enzymes, metallothioneins, DNA damage, and liver somatic index were observed in EFPC fish. In addition, reproductive impairment was observed in fish collected near the Y-12 Plant but not in fish collected ≥4 km farther downstream.

Instream monitoring of benthic macroinvertebrates indicate that species richness, diversity, density, biomass, and production were lowest at upstream sites. All of these parameters gradually increased with distance downstream. Although effluents released from the Y-12 Plant may account for the observed degradation of macroinvertebrate communities, the specific constituents causing the impacts have not yet been identified. The impacts probably result from a combination of several factors, such as elevated temperature, sublethal concentrations of one or more toxicants, episodic releases of toxicants, excess nutrients, organic loading, and/or siltation.

Instream monitoring also indicated that fish populations in EFPC are in poor condition, although some downstream recovery was observed. The Index of Biological Integrity (IBI) ratings for the upper reaches of EFPC were low but indicated slight improvement through time (i.e., upper reach ratings have increased from very poor to very poor bordering on poor). At downstream sites the IBI rating was very poor to poor initially, but showed steady improvement over the two-year sampling period. The slight improvements in the IBI ratings were supported by increases in the number of darter species, occurrence of intolerant species, and number of lithophilic spawners in lower EFPC.

The biological monitoring program has been effective in identifying contaminants of concern and providing information on contaminant spatial and temporal distribution and effects. Biological

monitoring has provided a successful and innovative alternative to water quality standards and/or toxicity testing during the initial stages of environmental restoration.

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MFO MEASUREMENTS IN ENVIRONMENTAL REGULATION. R.F. Addison, Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Nova Scotia.

Laboratory and field studies carried out during the last twenty years or so have shown the potential of fish hepatic MFO measurements as a screening procedure for the presence and effects of some organic contaminants. The incorporation of MFO measurements in monitoring programmes presents special problems, particularly in providing intercalibration standards and interpreting field data. These subjects will be discussed, particularly in light of experience with IOC/ICES monitoring excercises in Europe.

COMPARISON OF WILD FISH POPULATIONS BEFORE AND AFTER SECONDARY TREATMENT OF BLEACHED KRAFT MILL EFFLUENT.

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Abstract

Up until the initiation of secondary treatment in October of 1989, Jackfish Bay, Lake Superior, had received the primary-treated effluent from a bleached kraft mill for several decades. White sucker (Catostomus commersoni) collected in 1988 and 1989 exhibited an increased condition factor and an apparent increase in body fat, despite a decreased growth rate and gonadal size. The fish showed increased levels of hepatic MFO activity (metabolism of benzo(a)pyrene, diphenyloxazole and ethoxyresorufin) and decreased levels of gonadal steroids (testosterone, 11-ketotestosterone, 17ß-estradiol and 17α,20ß-dihydroxy-4-pregnen-3-one). Despite the lower steroid levels, and the reduction in secondary sex characteristics and gonadal size, there were no differences in fecundity, fertilization rate, hatch, survival or developmental rate of larvae. In comparison, Jackfish Bay lake whitefish (Coregonus clupeaformis) showed no evidence of gonadal maturation two months prior to the spawning season. Current studies are examining white sucker and lake whitefish for evidence of improvement after installation of a secondary treatment system, and have evaluated other species of fish for evidence of impacts. Studies conducted during 1990 and 1991 have confirmed impacts on maturity, MFO activity, serum steroids and age distribution in longnose sucker (Catostomus catostomus), and have failed to document improvement in white sucker or lake whitefish.

Introduction

Results from studies conducted during 1988 and 1989 at Jackfish Bay, Lake Superior, indicated that white sucker exposed to bleached kraft pulp mill effluent (BKME) exhibited a wide variety of impacts (McMaster et al., 1991a,b; Munkittrick et al., 1991a). The impacts found were very similar to those reported from Scandinavia during the early 1980's (reviewed in Sodergen, 1989), including delayed sexual maturity, smaller gonads, reduced body size, increased liver size and elevated mixed function oxygenase (MFO) activity.

The bleached kraft mill in Terrace Bay, Ontario, produces 1200 air dried metric tonnes (ADMT) of pulp per day, and has released primary treated effluent into Jackfish Bay for several decades. In October of 1989, the mill installed a secondary treatment aeration lagoon system. Since initiation of the lagoon operation, there have been substantial reductions in release of suspended solids, phosphorus and AOX, reduction of BOD by more than 95% (Environment Canada 1989; Ontario Ministry of Environment 1988; Environment Ontario 1991) and elimination of acute lethality of the effluent to fish caged in Jackfish Bay (K. Flood, Ontario Ministry of Environment, Toronto, ON, Canada M4V 1P5; unpubl. data). Conditions in Jackfish Bay, including water clarity and temperature of the discharge have also improved dramatically.

The 1988 and 1989 collections of white sucker represent detailed baseline studies on which to evaluate the performance of secondary treatment in mitigating the impacts of BKME on fish populations. The present studies have continued observations at Jackfish Bay during 1990 and 1991. Data describing lake whitefish (Coregonus clupeaformis) collections from 1989 and 1990 and longnose sucker

(Catostomus catostomus) during 1990 are presented elsewhere (Munkittrick et al. 1991b,c). This manuscript describes four years of data on white sucker collected from the spawning runs in streams at both Jackfish Bay (BKME) and Mountain Bay (reference).

Materials and Methods

Jackfish Bay, located along the north shore of Lake Superior, receives BKME from a mill located in Terrace Bay, Ontario. The pulp mill discharges approximately 121,000 m^3 d^{-1} of effluent into the headwaters of Blackbird Creek. The creek receives little dilution between the discharge from the mill, prior to its entry into Moberley Bay (48°50'N, 86°58'W), the western arm of Jackfish Bay, a distance of approximately 15 km. Over the last 40 years Moberley Bay has received either untreated or primary-treated effluent. The mill began operation in 1948 and installed two primary treatment clarifiers during expansion in 1978. Moberley Bay receives no other industrial or municipal effluents, making it an ideal site for studying the impact of BKME on fish populations.

White sucker from Jackfish Bay spawn in tributaries to Jackfish Lake, the only suitable spawning streams within the Jackfish Bay drainage basin. White sucker were captured from Sawmill Creek, an uncontaminated tributary of Jackfish Lake; the duration of residency in clean water prior to spawning is unknown and probably varies with weather conditions prior to and during the spawning season. For comparison, white sucker were collected from a spawning run in the Little Gravel River, a tributary to Mountain Bay, Lake Superior (48°56'N, 87°50'W). This reference site has been used previously to monitor impacts of BKME on Jackfish Bay fish populations (McMaster et al., 1991a; Munkittrick et al., 1991a; Smith et al., 1991) as it receives no industrial effluent or significant domestic effluent input.

Sampling Procedures

Prespawning white sucker were collected from spawning streams using overnight hoop net sets in mid-May. Female fish were separated from males and kept in aerated holding tanks filled with stream water until sampling. Male fish were placed in holding nets and left in the streams until immediately prior to sampling. Female fish were sampled between 1030 and 1400 h and male fish from 1500 to 1830 h to minimize variation in blood parameters. All fish were collected early in the run, and sampling procedures and collections were identical between sites.

Each fish was rendered unconscious by a sharp blow to the head. Fork length (mm) and total weight (g) were determined, and each fish was examined for external lesions. Blood was collected via caudal severance into 5.0 mL heparinized vacuum tubes, stored on ice for 6 to 8 h, and centrifuged prior to the collection of plasma. The liver was excised, weighed (± 0.1 g) and sampled for measurement of MFO activity. Both the plasma and liver samples were immediately frozen in liquid nitrogen and returned to the laboratory where they were stored at -80°C pending analysis.

Fish were aged by counting annuli on dried, cleaned opercula from each fish. The gonads were removed and weighed (± 0.1 g), and the ovaries preserved in 10% buffered formalin. In the laboratory, the preserved ovaries were washed, blotted dry and reweighed (± 0.001 g). The number of eggs in replicate, 1 g samples was determined and these results were used to estimate the total number of eggs per fish (total fecundity).

Testosterone and 17β-estradiol were measured by radioimmunoassay (RIA) in plasma following ether extraction. A description of the antisera used to measure testosterone and 17β-estradiol were reported in Van Der Kraak et al. (1984, 1990) and the RIA protocols are described in Van Der Kraak et al.

(1984). All plasma samples were assayed in duplicate and interassay variability was less than 15% in both RIA systems.

Results and Discussion

There was dramatic consistency in results from year to year. Most groups of fish showed less than 5% variability in length and weight between years, except for reference females which showed a lower weight during 1991 collections (Table 1). This may have been due to a very early spawning run, and our failure to collect the first fish ascending the stream, as had been done in all other years.

BKME-exposed fish showed consistent increases in weight and age, and no difference in length or fecundity between sites. There were no trends towards recovery in any of these parameters after initiation of secondary treatment. There has been no change in the age distribution of white sucker participating in the spawning run since secondary treatment (Figure 1). A delayed age to maturation has been reported for both lake whitefish (Munkittrick et al. 1991c) and white sucker (McMaster et al. 1991a; Munkittrick et al. 1991a) in Jackfish Bay. Longnose sucker also show an altered age distribution (Munkittrick et al. 1991b).

The most dramatic difference between sites has been in gonad weights, with Jackfish Bay females showing approximately 25 % smaller gonads. If gonad weight indicated any immediate recovery after secondary treatment, an increase in the frequency of larger gonads would be expected. Comparison of gonadosomatic index (GSI) frequency distributions over the 4 years of collections shows no trends towards recovery (Figure 2).

Jackfish Bay white sucker also show decreased secondary sex characteristics, in addition to the delayed maturity and reduced gonadal sizes. These are related to decreased levels of circulating sex steroids. All three species examined show depressed levels of sex steroids, during all seasons, but show dramatic differences between species in response to the lower levels of steroids. Although steroid difference are consistently three-fold or higher, white sucker show 25% smaller gonads, lake whitefish >50% smaller gonads, and longnose sucker show no differences in gonad size.

The cause of the steroid reduction is related to altered regulation, production and circulation of sex steroids (reviewed in Munkittrick et al. 1991d). Prespawning white sucker

a) do not respond to administration of gonadotropin-releasing hormone analogues by increasing production of 17α20β-dihydroxy-4-pregnen-3-one,

b) show lower levels of in vitro steroid production by prespawning eggs, and the steroid production is unresponsive to gonadotropin administration, and

c) circulating levels of steroids show very low levels of glucuronated forms.

There has been no evidence of recovery of circulating steroid levels since secondary treatment (Table 2), and there has been no improvement in in vitro steroid production between 1990 and 1991 (unpublished data).

All three species of fish also show marked induction of hepatic mixed function oxygenase (MFO) enzymes (Munkittrick et al. 1991b) as indicated by a number of different catalytic assays. During the summer period, EROD activity (ethoxyresorufin-o-deethylase) shows as much as 20-fold induction in white sucker. However, at spawning time, induction has been inconsistent (Table 3).

Table 1. **Interlaboratory EROD methodology comparison (labs 4 and 6 lost their samples prior to analysis).**

Lab	Buffer	pH	Mg^{++} mM	BSA mg mL[1]	NADPH mM	7-ER µM	Carrier	Protein mg/mL	Protein Method	Volume	Reaction Mixture (1,2,3,4,5,6)[1]	Time min	Temp °C
1	0.1 M HEPES	7.8	9	1.4	1.4	1.3	DMSO	0.6	Lowry	1.41	1250,10,50,30,20,50	4	25
2	0.1 M HEPES	7.8	17	1.4	0.5	1.7	DMSO	0.8	BioRad	1.43	1250,20,50,30,30,50	10	25
3	0.1 M HEPES	7.8	10	1.7	1.0[2]	1.6	DMSO		Lowry	1.25	1110,10,50,30,10,50	2	25
5	0.1 M KH_2PO_4	8.0	-	-	0.12	0.26	PO_4 buffer	0.4	Biorad	2.0	1690,0,0,10,100,200	10	30
7	0.1 M TRIS + 0.1 M NaCl	8.0	6.3	1.0	0.77[2]	2.0	MeOH	0.1	Lowry	1.0	640,0,100,50,10,200	15	37
8	0.1 M TRIS	8.5	-	-	0.008	1.0	TRIS/DMSO		BioRad	2.0	0,0,0,20,1970,10	2	37
9													
10	0.1 M HEPES	7.8	10.9	1.8	0.5	1.5	DMSO	0.8	BioRad	1.14	1000,10,50,20,10,50	2	25
11	0.1 M TRIS + 0.1 M NaCl	8.0	-	-	0.5	2.0	???	3 - 8					25
12	0.1 M HEPES	7.8	9	1.4	0.7	0.9	DMSO		Biuret	1.4	1250,10,50,30,10,50	2	25
13	0.05 TRIS	8.0	90	-	0.56	0.25	TRIS		Lowry	2.0	1820,0,0,30,100,50	10	25
14	0.05 TRIS/ sucrose	7.5	-	-	0.15	1.6	???	0.05	Lowry	1.25	1060,0,0,125,15,50	15	27

[1] Buffer, Mg, BSA, NADPH, ER, Protein in µL

[2] NADPH generating system, calculated as maximum NADPH

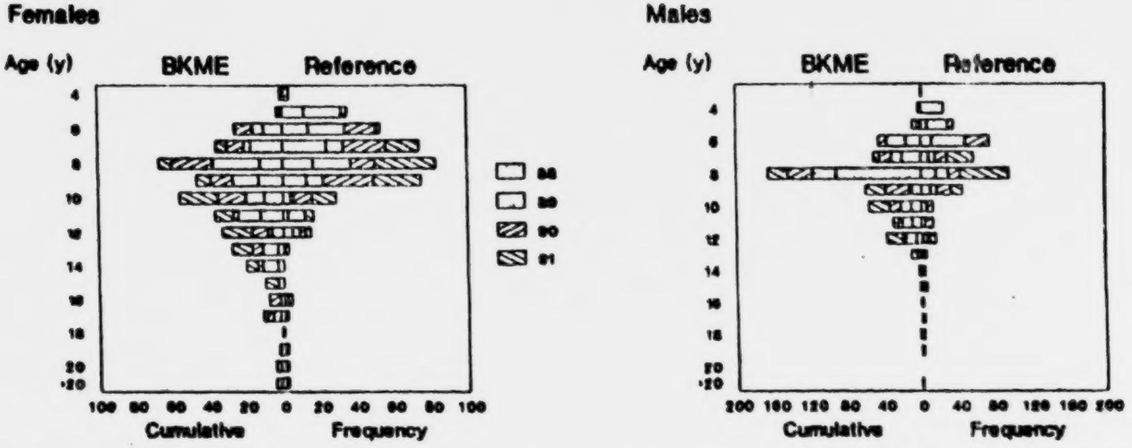

Figure 1. Cumulative frequency distribution for ages of female and male white sucker collected from spawning runs during 1988 - 1991. The absence of a shift in age distribution since 1989 (initiation of secondary treatment at Jackfish Bay) suggest there has been no recovery so far.

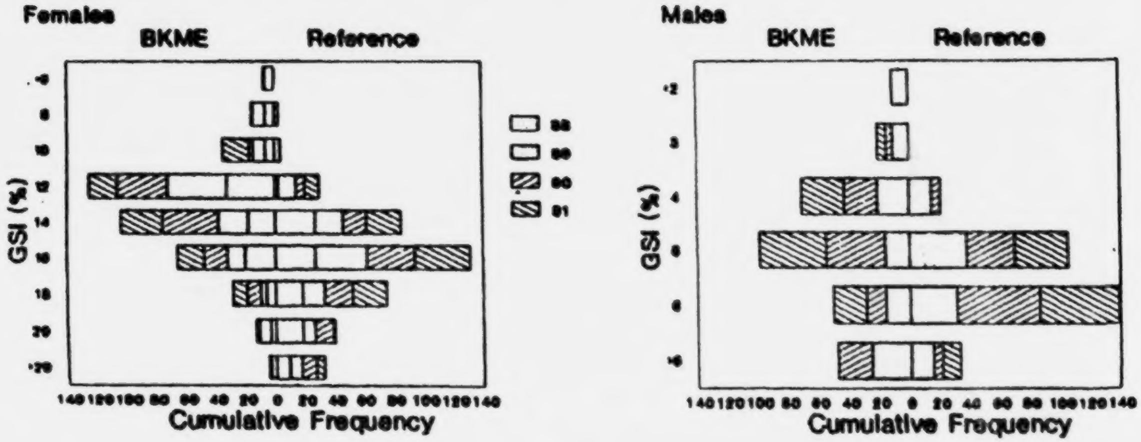

Figure 2. Cumulative frequency distribution for GSI (100 x gonad weight / (body weight - gonad weight)) of female and male white sucker collected from spawning runs during 1988 - 1991. The absence of a shift in GSI distribution since 1989 (initiation of secondary treatment at Jackfish Bay) suggest there has been no recovery so far.

Table 2. Levels of circulating sex steroids (pg mL^{-1}) in prespawning white sucker collected during 1989-1991 (no blood steroids were measured in 1988 prespawning fish; some 1991 samples still to be analyzed).

Sex	Site	Year	Testosterone	17β-estradiol	11-ketotestosterone	17α,20ß-dihydroxy-4-pregnen-3-one
Male	Jackfish	1989	2,798 ± 383 (10)**[1]		50,019 ± 7,991 (10)**	611 ± 48 (10)**
		1990	1,250 ± 141 (21)**			
		1991	1,753 ± 319 (6)**		13,054 ± 1,917 (6)**	589 ± 209 (6)
	Mountain	1989	7,661 ± 1,069 (10)[1]		107,849 ± 8,446 (10)	1,233 ± 257 (10)
		1990	5,481 ± 141 (10)			
		1991	3,330 ± 307 (6)		28,025 ± 3443 (6)	676 ± 190 (6)
Female	Jackfish	1989	4,848 ± 952 (10)**[1]	1,956 ± 449 (10)		463 ± 59 (10)
		1990	3,933 ± 679 (17)**			
	Mountain	1989	13,777 ± 4030 (10)	1,787 ± 329 (10)		448 ± 47 (10)
		1990	11,237 ± 2343 (12)[1]			

* significantly lower than reference site (0.05), ** (0.01)

[1] data from McMaster et al. (1992a)

Table 3. Hepatic MFO activity in prespawning white sucker.

Sex	Site	Year	MFO Activity	Sex	Site	Year	MFO Activity
Male	Jackfish	1988[1 2]	4.7 ± 0.6 (9)	Female	Jackfish	1988	1.5 ± 0.2 (11)
		1989[1 3]	6.89 ± 1.74 (6)**			1989	1.74 ± 0.54 (6)
		1990[4]	25.89 ± 4.3 (15)**			1990	2.47 ± 0.73 (12)
		1991[4]	22.4 ± 8.9 (7)**			1991	7.9 ± 3.4 (8)**
	Mountain	1988	4.5 ± 0.6 (11)		Mountain	1988	1.7 ± 0.3 (9)
		1989	3.20 ± 0.44 (6)			1989	0.76 ± 0.13 (6)
		1990	9.39 ± 1.39 (8)			1990	0.99 ± 0.51 (13)
		1991	2.34 ± 0.40 (11)			1991	0.85 ± 0.27 (13)

[1] Activity measured as AHH towards Benzo(a)pyrene

[2] data from Munkittrick et al. (1992a)

[3] data from McMaster et al. (1991)

[4] activity measured as EROD

It is not known if the absence of induction is related solely to migration to clean water, or whether some absence is attributable to a decrease in the activity of enzymes at spawning time. McMaster et al. (1991a) showed that levels in spawning fish were lower than prespawning fish, but this may have been due to a longer residence in clean water. Samples collected from the plume in Jackfish Bay during the 1991 spawning run, found very low numbers of sexually mature fish which showed no induction, and larger numbers of immature fish which showed induction (unpubl. data).

It is not known whether a relationship exists between MFO induction and circulating levels of sex steroids. Steroid disruption is more persistent than MFO induction. Steroidal problems are still evident during spawning, although MFO induction is lower. Furthermore, EROD activity declines very rapidly during shutdown, but depressed steroid levels are still evident (Munkittrick et al. 1991b). The disappearance or reduction of MFO activity during mill shutdown (Munkittrick et al. 1991b) suggests that secondary treatment has not removed the chemicals responsible for MFO induction, and that contamination is not occurring solely via historical sediment contaminants.

There have been no trends towards improvement in MFO activity, blood steroid levels, gonadal size, growth rate or age distribution during the first 18 months of secondary treatment.
Condition factor appears to have shown recovery in male fish. The increased condition factor during primary treatment was correlated with a decreased presence of visceral lipids, which has not shown evidence of recovery (unpublished data). It is clear that recovery of Jackfish Bay will not be rapid, although it is too soon to predict if Jackfish Bay fish populations will recover with the process and treatment changes undertaken at Terrace Bay.

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EVALUATION OF BIOINDICATORS OF CONTAMINANT-EXPOSURE AND EFFECTS IN COASTAL ECOSYSTEMS. U. Varanasi, J.E. Stein, T.K. Collier, L.L. Johnson, E. Casillas and M.S. Myers, National Marine Fisheries Service, NOAA, Seattle, Washington.

Contamination of coastal environments by anthropogenic chemicals has led to increased efforts to understand relationships between contaminant exposure and biological effects in aquatic organisms. These efforts have identified a need for indicators of both contaminant exposure and early events in contaminant-induced diseases or dysfunction that can be readily measured in feral animals. In contaminated environments organisms are exposed to complex mixtures of chemicals, many of which are not identified; thus, procedures are needed that can measure both exposure and effects by a variety of different chemicals. In a series of studies to investigate potential advantages from the use of a suite of bioindicators for assessing environmental quality, we have measured indices of contaminant exposure [hepatic polychlorinated biphenyls (PCBs) and levels of fluorescent aromatic compounds in bile, a measure of exposure to aromatic hydrocarbons (AHs)] and biochemical and biological effects (hepatic monooxygenase (MO) activities, hepatic glutathione levels, hepatic DNA-xenobiotic adducts, levels of estradiol and vitellogenin in plasma, histological assessment of ovarian maturation, spawning and fertilization success, larval viability and pollution-associated liver lesions) in several benthic flatfish from sites in Puget Sound, WA, which ranged from highly contaminated to minimally contaminated. Both juvenile and adult fish were used in these studies. The results with adult female English sole (>300mm) showed that when exposed to high levels of AHs and PCBs in their environment these fish were less likely to undergo ovarian maturation than those residing in minimally-contaminated areas and that fish from heavily contaminated sites also had lower levels of estradiol in the blood. Both inhibited ovarian maturation and depressed plasma levels showed a strong association with elevated hepatic MO activity, a sensitive biomarker of contaminant exposure. Also, an inverse relationship between levels in plasma estradiol and subsequent spawning success was observed. The successful use of certain biomarkers to evaluate the impact of contaminant exposure on reproductive processes in English sole suggest the potential utility of such an approach with other fish species. Moreover, the studies on juvenile of three species of flatfish also demonstrated the usefulness of hepatic MO activity as a bioindicator, as well as demonstrating the potential of hepatic DNA-xenobiotic adducts, measured by the ^{32}P-postlabeling assay, and glutathione levels as indices of exposure and sublethal effects in feral fish. Overall, the results of these studies showed that there was general concordance between most bioindicators and the degree of chemical contamination at the site, although specific instances of a lack of correlation were noted for some indicators. Hence, the results suggest that a more comprehensive assessment of the impact of a complex mixture of contaminants on feral fish can be attained through a suite of bioindicators, rather than one or two tests.

SESSION 3C

BIOLOGICAL APPROACHES TO SITE-SPECIFIC EFFLUENT REGULATION/APPROCHES BIOLOGIQUES À LA REGLEMENTATION DES EFFLUENTS À DES ENDROITS DONNÉS

CHAIRPERSON/PRÉSIDENT
John Sprague

LES CRITÉRES QUALITÉ DE L'EAU AU QUÉBEC: DÉFINITIONS ET APPLICATION.

I. Guay, Service d'évaluation des rejets toxiques, ministère de l'Environnement du Québec, Québec.

RÉSUMÉ

Le ministère de l'Environnement du Québec (Menviq) a récemment rendu publics deux documents concernant les critères de qualité de l'eau qu'il entend utiliser pour des fins de suivi de qualité et de protection des usages du milieu récepteur aquatique. Le Menviq a ainsi retenu des critères de l'eau pour plus de trois cents contaminants, pour la plupart des substances toxiques, ainsi qu'une méthodologie permettant de calculer un critère pour les substances potentiellement nuisibles qui n'en possèdent pas encore.

Les critères de qualité de l'eau reflètent la meilleure information disponible jusqu'à présent sur les effets délétères d'un contaminant. L'utilisation de ses valeurs ne peut se faire sans l'application constante du "meilleur jugement professionnel". Il est donc nécessaire que leurs utilisateurs connaissent les grandes lignes de leur fondement ainsi que leur cadre d'application.

Dans ce contexte, le présent exposé a pour but de donner les définitions des critères, d'expliquer leurs fondements scientifiques et surtout d'identifier leurs limites d'application tant techniques qu'administratives. L'emploi qu'en fait actuellement le Menviq sera aussi abordé.

ABSTRACT

The ministère de l'Environnement du Québec (MENVIQ) recently published two documents concerning the water quality criteria it intends to use to monitor and protect receiving water bodies. MENVIQ has selected water quality criteria for over three hundred contaminants, mostly toxic substances, and adopted methods enabling it to set criteria for possible harmful substances for which such criteria do not yet exist.

Water quality criteria provide the best information available on the harmful effects of contaminants, and highest professional judgment must be used constantly in their application. Persons applying these criteria must therefore be familiar with their basic premises and the framework of their application.

The purpose of this presentation is to provide definition of water quality criteria, explain their scientific basis and, in particular, identify restrictions in terms of their technical and administrative application. MENVIQ's use of these criteria will also be discussed.

INTRODUCTION

Outils complémentaires aux bioessais sur effluent entier et aux bioindicateurs dans le milieu, les critères de qualité de l'eau sont de plus en plus utilisés par les organismes gouvernementaux, les consultants, les chercheurs, pour des besoins de gestion, d'évaluation ou de comparaison. Malheureusement, il arrive parfois que les valeurs sont mal utilisées car leurs utilisateurs en connaissent mal les fondements et les limites d'utilisation. Par ailleurs beaucoup d'organismes publient sans cesse de nouveaux critères, normes, recommandations, etc.; il est alors essentiel d'aider les utilisateurs à en comprendre les nuances.

Les critères de qualité spécifiques sont toujours des outils essentiels au contrôle des rejets toxiques et à l'évaluation générale de la qualité des eaux:

- parce qu'ils demeurent presque le seul outil préventif pour une nouvelle substance;
- parce qu'ils tiennent compte, bien que de façon prédictive, d'effets sur la santé tels la cancérogénécité potentielle des substances, les autres effets toxiques chez l'humain, etc.;
- parce qu'ils peuvent mettre en évidence une toxicité cachée due au relargage, à la mise en disponibilité lors de la dilution d'une substance qui était complexée dans l'effluent, de la transformation de substances moins toxiques en produits plus toxiques lors de la dégradation de l'accumulation dans les sédiments, etc..

L'utilité des critères de qualité n'étant donc plus à défendre, le Ministère devrait alors s'assurer de l'uniformité des critères utilisés à travers le Québec. C'est ce qu'il a fait en publiant récemment un document contenant un inventaire de critères de qualité de l'eau ainsi q'une méthode permettant d'en déterminer pour de nouvelles substances. Maintenant il en est rendu à s'assurer de la bonne utilisation de ces critères.

Cette présentation vise donc principalement les intervenants des milieux public et privé, des organismes environnementaux, ou autres qui ont ou qui auront à évaluer la qualité des eaux, à gérer des sources polluantes ou à conseiller des utilisateurs non-initiés. Tous devraient se familiariser avec les grandes lignes qui ont permis d'établir de telles valeurs, connaître les avantages des critères spécifiques et nécessairement connaître leurs limites.

L'exposé sera composé d'un bref survol historique indiquant le contexte qui a amené le Menviq à se doter de ces outils en fonction des mandats qui lui étaient assignés, de la présentation des critères de l'eau existants au Québec, pour finalement s'orienter vers le cadre d'application des critères.

HISTORIQUE

Le ministère de l'Environnement du Québec (MENVIQ) est chargé d'identifier les contraintes pour la protection de la santé humaine et des ressources biologiques dans une optique de maintien et de récupération des usages de l'eau et des ressources biologiques aquatiques. Dans ce contexte, notre besoin était et est encore de définir les niveaux d'intervention pour les sources pontuelles de pollution à partir de la qualité nécessaire au maintien ou à la récupération des usages de l'eau. Il fallait alors en premier lieu définir quels étaient les usages potentiels de l'eau (tableau 1) puis déterminer la qualité idéale pour le maintien de ces usages. Cette qualité idéale est représentée par les critères de qualité de l'eau. On entend alors par critère une concentration seuil uniquement basée sur des contraintes environnementales (toxicité, organolepticité, esthétique), dont le dépassement risque d'entraîner la perte complète ou partielle de l'usage auquel elle correspond.

Puisqu'il existait déjà bon nombre de valeurs publiées (depuis au moins 1963), l'approche adoptée a consisté à recenser et à analyser les critères, recommandations, objectifs, lignes directrices, etc. correspondant à un seuil sécuritaire pour un usage spécifique provenant des publications de la Direction de la qualité des eaux d'Environnement Canada, de Santé et Bien-être social Canada, des ministères des provinces canadiennes, de la Commission mixte internationale (CMI), de la United States Environmental Protection Agency (USEPA) ainsi que des organismes de protection de l'environnement des États américains, de la Commission européenne consultative pour les pêches dans les eaux intérieures (EIFAC), de l'Organisation mondiale de la santé (OMS), de la Communauté économique européenne (CEE) et finalement du Conseil canadien des ministres de l'Environnemenmt (CCME). Au-delà de 3000 valeurs ont été recensées.

Tableau 1: Principaux usages de l'eau

.	Source d'eau potable:	Alimentation Usages domestiques
.	Pêche et consommation d'organismes aquatiques	
.	Vie aquatique:	Faune, flore
.	Vie terrestre:	Source d'eau Source alimentaire
.	Activités récréatives:	À contact primaire À contact secondaire
.	Autres:	Irrigation Abreuvement du bétail Prises d'eau industrielles

Puisque pour une même substance et un même usage, il existait parfois plus d'une valeur, il fallait faire un choix. Pour ce faire, il était nécessaire de très bien connaître le fondement de chacune des valeurs: ce qui a permis de fonder ce choix sur l'approche possédant les "meilleures" justifications environnementales et toxicologiques. Le choix d'une méthode de calcul de critères de qualité pour les substances toxiques s'en est suivi. La sélection des valeurs de critère s'est faite par la suite le plus possible en fonction de la méthode de calcul adoptée. Ces deux étapes: choix d'une méthode et sélection des critères ont donné naissance aux deux documents présentés dans les pages qui suivent.

PRÉSENTATION DES CRITÈRES DE QUALITÉ DE L'EAU AU QUÉBEC

Deux documents concernant les critères de qualité de l'eau ont été officiellement publiés en mai 1991 soit "Critères de qualité de l'eau" (Menviq, 1990a) et "Méthode de calcul des critères de qualité de l'eau pour les substances toxiques" (Menviq, 1990b).

Critères de qualité de l'eau

Ce premier document est donc l'inventaire des critères de qualité existant dans d'autres juridictions et pour lesquels une sélection a été faite lorsque cela s'avérait nécessaire. Des critères ont été ainsi retenus pour plus de 300 substances. Pour chaque substance, 6 niveaux de contraintes permettent de classer les critères selon l'endroit où ils doivent être appliqués dans le milieu (tableau 2). Pour la vie aquatique, des critères distincts ont été retenus pour les eaux douce et salée.

Tableau 2: Niveaux de contraintes identifiés pour l'application des critères de qualité de l'eau

Santé humaine	
. Eau brute (prise d'eau domestique)	. toxicité (consommation d'eau et d'organismes aquatiques) . organolepticité . esthétique
. Contamination d'organismes	. toxicité (consommation d'organismes aquatiques)
Vie aquatique	
. Toxicité aiguë	. toxicité aiguë sur la faune aquatique
. Toxicité chronique	. toxicité chronique sur la faune et la flore aquatiques . toxicité chronique sur la faune terrestre . organolepticité de la chair de poisson
Activités récréatives	
. À contact primaire	. esthétique . organolepticité . toxicité
. À contact secondaire	. esthétique

Ce domaine étant en perpétuel développement, il y a sans cesse de nouvelles informations, de nouvelles valeurs et de nouvelles procédures qui s'ajoutent. Les valeurs contenues dans le document sont celles qui doivent être utilisées jusqu'à la prochaine mise à jour du document. Toutefois, pour certaines substances, seule une donnée de toxicité identifiée comme telle est présente dans le document. Le Service d'évaluation des rejets toxiques a parfois calculé des critères pour ces substances. Ils seront éventuellement et graduellement ajoutés au document.

Méthode de calcul des critères de qualité de l'eau pour les substances toxiques

Certaines substances qui n'ont pas encore été l'objet d'une publication officielle pourraient avoir un potentiel toxique, les tributyls étain dont les critères ont récemment été publiés en sont un exemple. C'est pourquoi l'absence de critères pour une substance n'implique pas nécessairement que cette dernière est sans effet ou sans danger pour l'usage concerné. Parmi les substances pour lesquelles aucun seuil sécuritaire n'a été défini jusqu'ici, plusieurs possèdent quelques données toxicologiques.

Dans ce contexte, le deuxième document a été rédigé afin de fournir une méthode permettant de maximiser l'utilisation des données toxicologiques présentes dans la littérature ou nouvellement générées, dans le but de définir des seuils pour les polluants ne possédant pas de critères publiés. La méthode d'élaboration de critères doit permettre la formulation de recommandations quantitatives sécuritaires nécessaires au contrôle préventif des rejets de polluants des sources ponctuelles. La méthode est donc basée sur une extrapolation de données toxicologiques connues et sur des facteurs de sécurité d'autant plus élevés que les données sont déficientes.

Ce 2^e document provient de la compilation et de l'analyse des diverses approches utilisées par l'Agence de protection de l'environnement fédérale américaine (U.S.EPA) ainsi que par certains États américains (Ohio, Michigan, Minnesota, Illinois et New York) et par le ministère de l'Environnement de l'Ontario. Dans son ensemble, l'approche adoptée par le MENVIQ s'apparente à l'approche du Département des ressources naturelles de l'État du Michigan (MDNR); cependant quelques améliorations proposées par d'autres organismes y ont été ajoutées. La méthode comprend plusieurs procédures distinctes permettant le calcul de critères en fonction des multiples effets d'une substance:

- Calcul de concentrations protégeant des effets toxiques: la vie aquatique, la faune terrestre et la santé humaine.

- Calcul de concentrations procurant un degré acceptable de risque de cancer pour les substances potentiellement cancérigènes.

- Calcul d'une concentration en deça des seuils perceptibles des goût, odeur, couleur de l'eau ou de la chair de poisson.

Critères de santé humaine

Les critères de santé humaine (CSH) sont établis dans le but d'estimer les concentrations qui ne présentent pas de risque pour la population si elles ne sont pas dépassées dans les eaux de surface. Plusieurs procédures ont été mises au point pour estimer ces concentrations. Elles dépendent principalement de l'effet produit par la substance; la procédure sera différente si elle produit un effet avec seuil ou un effet sans seuil. Un effet avec seuil sous-entend que l'organisme biologique possède une réserve physiologique devant être comblée avant l'apparition d'effets délétères. Pour les substances qui entraînent ce genre de réponse, il est possible de définir une dose en dessous de laquelle les organismes exposés ne subiront pas d'effets toxiques. L'effet sans seuil suppose que l'exposition d'un organisme à n'importe quelle concentration, si petite soit-elle, peut entraîner un effet délétère. Dans un but de prévention, les effets de cancérogénécité seront considérés comme des phénomènes sans seuil. Pour ces substances, une concentration associée à un niveau de risque acceptable est déterminée.

Indépendamment de la réponse induite par une substance (avec ou sans seuil), les critères de santé humaine tiennent compte de deux sources d'exposition à partir de l'eau, l'ingestion d'eau et d'organismes aquatiques. Pour les eaux de surface destinées à l'alimentation en eau potable, les CSH sont calculés de façon à protéger un individu qui consomme pendant toute sa vie une eau contaminée à cette concentration et qui consomme des poissons qui ont bioconcentré la substance à partir de l'eau contaminée à la concentration du CSH. Pour les eaux de surface ne servant pas de source d'eau potable, le CSH évite la contamination des organismes aquatiques jusqu'à des niveaux nuisibles à la santé humaine. Le critère de santé humaine qui tient compte de la consommation d'eau et d'organismes aquatiques s'appelle critère d'eau brute (destinée à la consommation domestique), et le critère de santé humaine qui tient compte uniquement de la consommation d'organismes s'appelle critère de contamination d'organismes aquatiques (COA).

Le CSH pour une substance non cancérigène doit être calculé à partir de données toxicologiques appropriées selon l'équation générale suivante:

$$\mathrm{CSH} = \frac{\mathrm{QMT} \times \mathrm{K}}{\mathrm{Vh} + (\mathrm{N} \times \mathrm{FBC})}$$

où: QMT = quantité maximale du toxique n'ayant aucun effet chez l'humain lorsque ingéré quotidiennement sur une base chronique, (mg toxique/j).

Vh = volume d'eau consommé quotidiennement (L/j).

N = quantité moyenne de chair d'organismes aquatiques consommée quotidiennement (Kg poisson/j).

K = pourcentage de l'exposition dû à l'ingestion d'eau et d'organismes aquatiques par rapport aux autres sources d'exposition.

FBC = facteur de bioconcentration (mg toxique/kg poisson divisé par mg toxique/L eau).

La QMT est calculée à partir de données toxicologiques, souvent sur des mammifères, et de facteurs de sécurité prenant en considération les variabilités intra et interspécifiques ainsi que la durée de l'exposition, etc. La QMT correspond aux doses de référence (DRF) contenues dans la banque de données IRIS. Les équations nécessaires à son calcul sont du type:

$$QMT = \frac{NOAEL\ (mg/kg) \times \frac{Na}{Pa} \times Ph}{FI}$$

où: Na = quantité de nourriture contaminée consommée quotidiennement par l'animal (kg)

Pa = poids de l'animal testé (kg)

Ph = poids d'un adulte (kg)

FI = facteur d'incertitude (10 à 10 000)

Critères de vie aquatique

Deux critères de vie aquatique sont déterminés pour assurer une protection à court et à long terme de tout le cycle de vie aquatique: le critère de toxicité aquatique aiguë et le critère de toxicité aquatique chronique. Le critère de toxicité aquatique chronique (CTAC) est la concentration la plus élevée d'une substance qui théoriquement ne produira aucun effet néfaste sur les organismes aquatiques (et leur progéniture) qui y sont exposés quotidiennement pendant toute leur vie. Le critère de toxicité aquatique aiguë (CTAA) est la concentration maximale d'une substance à laquelle les organismes aquatiques peuvent être exposés pour une courte période de temps sans qu'ils soient atteints sévèrement.

Les procédures utilisées pour calculer les critères de toxicité aquatique aiguë ou chronique dépendent du nombre de données aiguës ou chroniques existantes pour la substance. La procédure complète nécessitant un grand nombre de données de toxicité permet de formuler des critères finaux en autant qu'un critère puisse être final. Une procédure simplifiée nécessitant un moins grand nombre de données permettra de déterminer des critères provisoires sécuritaires à l'aide de facteurs interspécifiques tenant compte du peu d'espèces testées. Finalement, dans certains cas il pourra s'avérer nécessaire de calculer des critères préliminaires à partir de quelques données seulement et de facteurs de sécurité, surtout dans le but de mettre en évidence le manque d'information flagrant sur certaines substances toxiques.

La procédure permettant le calcul d'un critère final nécessite la disponibilité de données représentant au moins 6 familles d'organismes aquatiques. Elle est basée sur l'extrapolation des données de toxicité aiguë (ou chronique) en une concentration suffisamment sécuritaire pour protéger 95 % (5e percentile) des espèces du milieu récepteur (figure 1). La valeur A ainsi trouvée pourra subir encore une transformation avant d'être appelée critère (Menviq, 1990b).

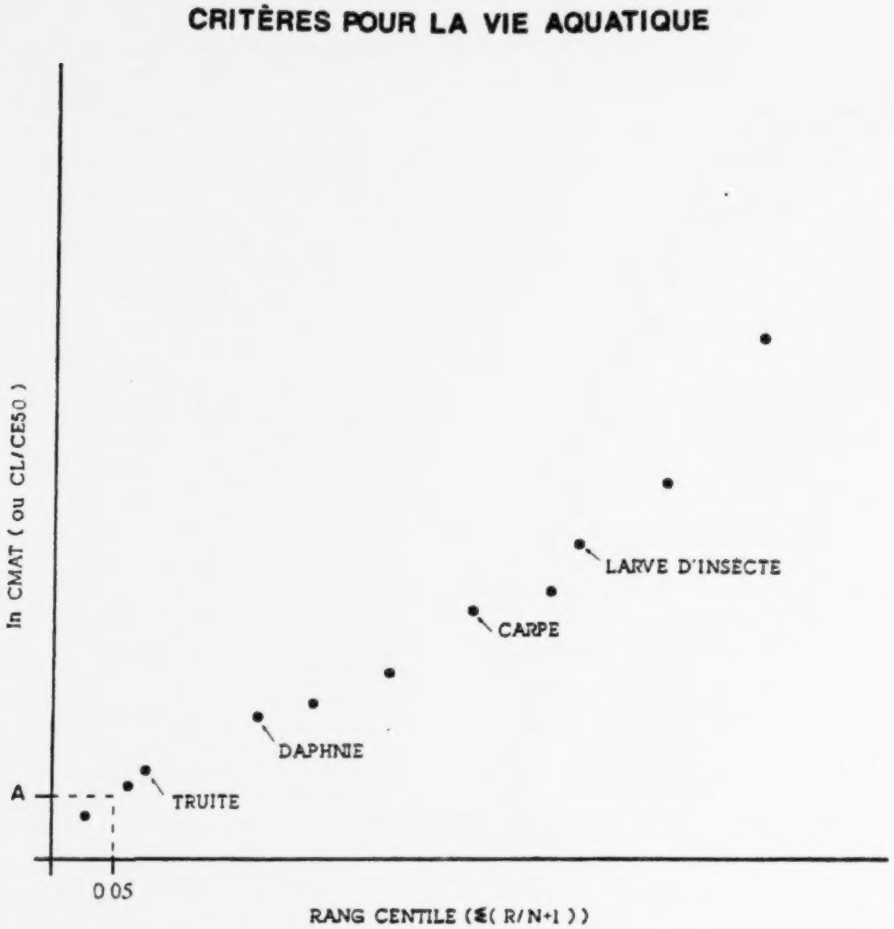

Figure 1: Représentation schématisée du calcul de la valeur aiguë ou chronique finale nécessaire au calcul d'un critère de vie aquatique

Valeur aiguë (ou chronique) finale = e^A

La méthode de calcul de critères pour la vie aquatique a déjà été employée dans quelques cas spéciaux pour des substances susceptibles de se retrouver à des effluents industriels (tableau 3). Elle est aussi employée pour le calcul de critères de substances toxiques pour lesquelles l'U.S.EPA n'a publié que des données de toxicité jusqu'à maintenant (tableau 4). Le calcul de critères sera priorisé en fonction de la non-biodisponibilité d'un critère de contamination d'organismes aquatiques pour une substance et de leur détection dans un ou plusieurs effluents.

Tableau 3: Substances pour lesquelles des critères provisoires pour la vie aquatique ont été calculés par le Service d'évaluation des rejets toxiques du Menviq

Acide styphnique	(1990)	Formaldéhyde	(1988)
Acrylamide	(1989)	Glyoxal	(1990)
Alkylbenzène linéaire	(1991)	Isophorone	(1991)
Bisulfite de sodium	(1989)	Morpholine	(1990)
Dichlorocyclohexane, 1,2-	(1990)	Nitrobenzène	(1991)
Dichloroéthane, 1,2-	(1989)	Nitroglycérine	(1991)
Dichloroéthylène, 1,1-	(1989)	Phosphate de tributyl	(1990)
Dinitrobenzène, 1,3-	(1988)	Styrène	(1989)
Dinitrotoluène, 2,4-	(1988)	Thiocyanates	(1990)
Dinitrotoluène, 2,6-	(1988)	Trinitrobenzène, 1,3,5-	(1988)
Dinitrotoluène, 3,4-	(1988)	Trinitrotoluène, 2,3,6-	(1988)
Dinitrotoluène, 3,5-	(1988)	Trinitrotoluène, 2,4,6-	(1988)
Fluorures	(1989)		

Tableau 4: Substances pour lesquelles l'U.S.EPA n'a publié que des données de toxicité pour la vie aquatique

Acénaphtène	(provisoire, 1989)	Halométhanes	
Acrylonitrile		Hexachloroéthane	
Antimoine		Hydrocarbures aromatiques polycycliques	
Benzidine		Isophorone	(provisoire, 1991)
Chloroforme		Naphtalènes chlorés	(en cours)
Dichloroéthane 1,2-	(provisoire, 1989)	Nitrobenzène	(provisoire, 1991)
Dichloroéthylène 1,1-	(provisoire, 1989)	Nitrophénols	
Dichloropropanes		Nitrosamines	
Dichloropropènes		Pentachloroéthane	
Diméthylphénol 2,4		Tetrachloroéthane,1,1,2,2	
Diphénylhydrazine 1,2-		Tetrachloroéthène	
Ethers halogénés		Tetrachlorure de carbone	
Fluoranthène		Thallium	
		Trichloroéthane 1,1,2	(provisoire, 1989)

La méthode de calcul pour les critères de santé humaine (consommation d'eau et de poisson ou consommation de poisson uniquement) sera principalement utilisée pour mettre à jour les critères de l'U.S.EPA de 1980 (et 1984 dans le cas de la dioxine). Cette mise à jour se fera selon le schéma présenté à la figure 2.

Ces critères devraient tous être mis à jour d'ici la prochaine version du document possiblement entre mai et septembre 1992.

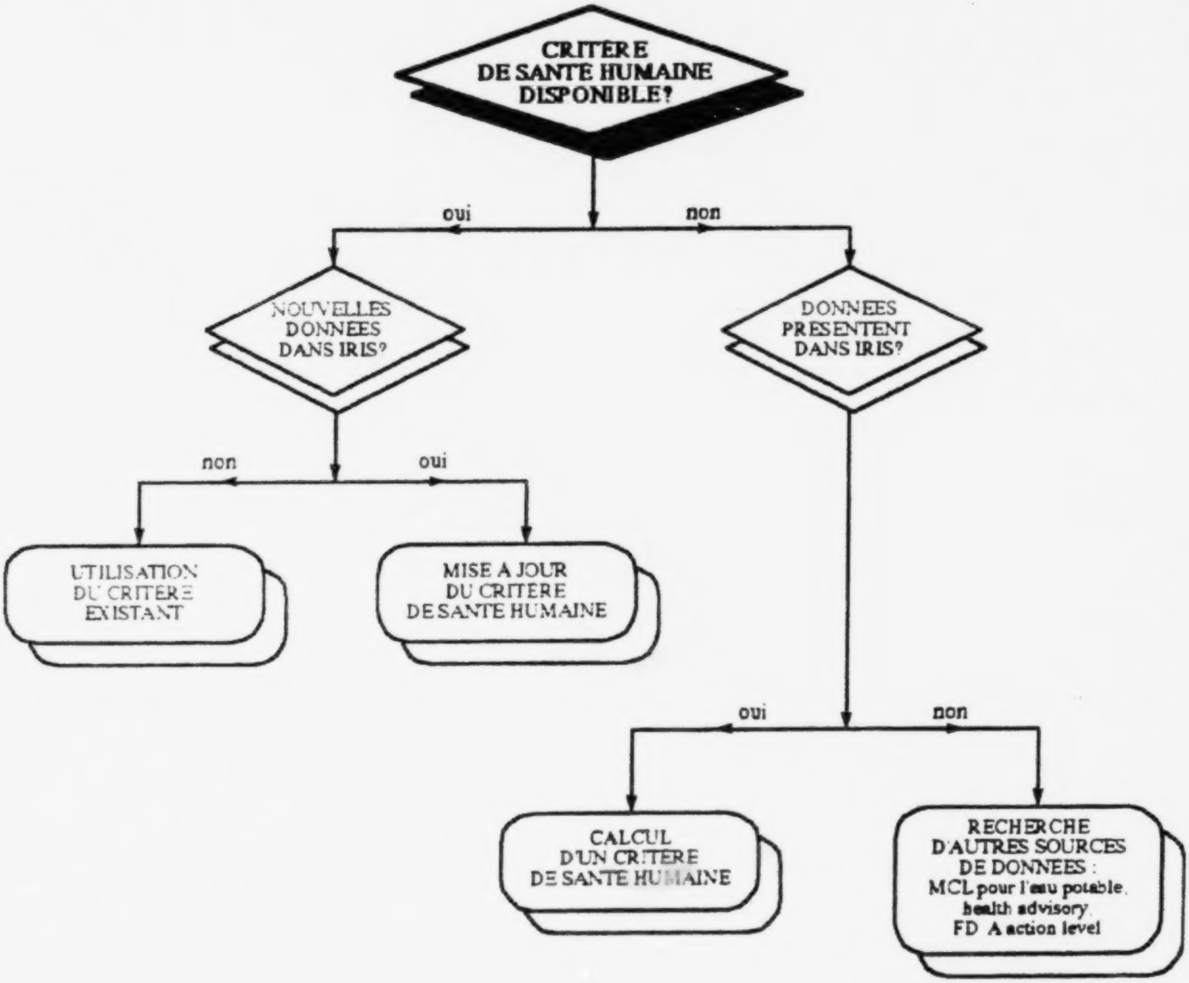
CRITERE DE SANTE HUMAINE DISPONIBLE?
oui
non
NOUVELLES DONNEES DANS IRIS?
DONNEES PRESENTENT DANS IRIS?
non
oui
UTILISATION DU CRITERE EXISTANT
MISE A JOUR DU CRITERE DE SANTE HUMAINE
oui
non
CALCUL D'UN CRITERE DE SANTE HUMAINE
RECHERCHE D'AUTRES SOURCES DE DONNEES : MCL pour l'eau potable, health advisory, FD A action level

Figure 2: Schéma de la procédure de mise à jour des critères d'eau brute et de contamination d'organismes aquatiques (modifié de U.S.EPA, 1990)

APPLICATION DES CRITÈRES POUR LES SUBSTANCES TOXIQUES

Le choix, l'utilisation et l'application des critères doivent toujours se faire selon le meilleur jugement professionnnel; l'utilisateur doit premièrement bien identifier le but visé pour être en mesure de choisir adéquatement le critère approprié et de l'appliquer selon son cadre d'application. L'utilisation abusive de ces valeurs a pu entraîner de mauvaises décisions.

Voici donc quelques mises en garde et quelques précisions quant au cadre entourant les critères de qualité de l'eau.

Critères versus normes

Les critères de qualité de l'eau ne sont pas des normes. Ces valeurs n'ont pas force de loi en tant que telles; elles s'intègrent dans des procédures globales où elles servent de base à la définition de niveaux d'intervention d'assainissement ou à l'évaluation de la qualité des eaux. Les critères de qualité sont des valeurs associées à un seuil sécuritaire protégeant un usage des effets délétères de tout ordre: toxicité, organolepticité, esthétique.

Les normes tiennent compte de l'état actuel des limites de détection des méthodes analytiques, des technologies de traitement et des coûts associés.

Eau brute versus eau potable

Les critères d'eau brute (consommation d'eau et d'organismes aquatiques) diffèrent des critères d'eau potable du fait justement qu'ils protègent deux usages simultanément dans un cours d'eau. C'est-à-dire qu'un individu pourra consommer de l'eau et des poissons provenant du même cours d'eau, ayant ainsi deux apports de toxiques directement de l'eau, sans effet sur sa santé. Le critère d'eau potable permettra une concentration plus élevée dans l'eau car il suppose que l'apport de toxique provenant du milieu aquatique vient uniquement de l'eau.

Critères de qualité pour l'eau salée

Les critères de santé humaine pour la consommation d'organismes aquatiques, les critères basés sur la toxicité pour la faune terrestre, ainsi que les critères organoleptiques et esthétiques peuvent s'appliquer aux eaux douce et salée jusqu'à ce que de nouvelles informations nous indiquent le contraire. Toutefois, pour la vie aquatique, des critères distincts ont été retenus ou définis pour chacun des types d'eau.

Critères spécifiques et critères de famille

Le meilleur jugement professionnel s'applique encore lorsqu'il s'agit de choisir entre un critère spécifique et un critère de famille (ex. fluoranthène ou HAP totaux). Le choix doit se faire en fonction de l'objectif visé et de l'usage concerné. Souvent les critères spécifiques sont définis à partir de données plus récentes; leur fiabilité en est alors améliorée. Toutefois, puisqu'il existe rarement des critères pour toutes les substances d'une famille, il est nécessaire d'utiliser aussi le critère de famille. L'utilisation d'un critère de famille implique que la somme des concentrations des substances qui la composent ou identifiées comme telles (ex. le critère de HAP totaux s'applique uniquement aux HAP considérés cancérigènes) ne dépasse pas ce critère de famille et qu'en plus, chacune des substances spécifiques ne dépasse pas son propre critère si celui-ci s'avère inférieur au critère total.

Lieu d'application des critères

Les critères de qualité sont utilisés entre autres pour l'élaboration d'objectifs environnementaux de rejet pour définir des niveaux d'assainissement pour les sources ponctuelles de pollution. Les critères concernant des usages présents ou potentiels à tous les plans d'eau sont appliqués à la limite d'une zone de mélange restreinte en tenant compte de la dilution de l'effluent dans le milieu. Ces usages sont ceux de vie aquatique et de faune terrestre qui lui sont associés ainsi que celui de consommation des organismes aquatiques qui sont basés sur des effets chroniques. Il faut cependant que cette zone de mélange soit exempte de toxicité aiguë pour les espèces aquatiques qui s'y trouvent ou qui auront à la traverser. L'effluent ne doit pas être toxique à court terme; les critères de toxicité globale (bioessai) aiguë s'appliquent à l'effluent et les critères spécifiques de toxicité aiguë en sont un indicateur. Finalement, les critères associés à la protection des sources d'eau potable et de récréation s'appliquent au site de l'usage (eg. prise d'eau, plage).

Intensité, durée et fréquence de dépassement

Pour éviter l'apparition d'effets aigus sur les organismes aquatiques, il est nécessaire de restreindre au minimum la durée d'exposition car la présence d'une substance toxique à une concentration supérieure au critère de toxicité aiguë peut entraîner un effet quasi instantané. Conséquemment les critères de toxicité aiguë doivent être respectés en moyenne pour des durées allant de 1 heure à 1 journée.

Un dépassement du critère chronique pour la vie aquatique pendant une période relativement courte peut entraîner un effet sur le biote aquatique même si en moyenne le critère de toxicité chronique n'est pas dépassé sur une plus longue période. Pour cette raison, les critères de toxicité chronique pour la vie aquatique doivent être respectés sur des périodes allant de 4 à 7 jours.

Les critères liés à la santé humaine et aux effets sur la faune terrestre sont fonction d'une durée d'exposition à long terme généralement la vie durant. Puisque les effets liés à une exposition continuellement au-dessus de la valeur du critère ne sont pas connus, la période moyenne généralement choisie pour le respect de ces critères est de 30 jours.

Caractéristiques physicochimiques du milieu récepteur

Les conditions environnementales influent de différentes façons sur la toxicité des polluants et plusieurs critères de vie aquatique devront prendre en compte les conditions locales du milieu récepteur (dureté, pH, température, quantité de matières organiques, etc.). Le choix de la valeur du paramètre physicochimique sera encore fonction de l'objectif visé. Généralement, on tentera de définir la période critique de façon à protéger le milieu en tout temps. Les valeurs représentatives liées aux critères de toxicité aiguë sont généralement déterminés à partir des caractéristiques de l'effluent tandis que celles liées aux critères de toxicité chronique le sont à partir des caractéristiques du milieu récepteur.

Devenir environnemental

- **Interactions entre les polluants**

  Les critères pour les contaminants spécifiques sont généralement comparés un à un à la qualité du milieu; les effets de synergie, d'additivité ou d'antagonisme provenant du mélange de plusieurs polluants ne sont pas pris en compte pour le moment par ce type de critère.

  Toutefois le respect simultané des critères de toxicité globale à l'aide d'un ou de plusieurs bioessais sur l'effluent entier nous permet de nous assurer de l'effet combiné des polluants sur la vie aquatique. Par ailleurs, l'adoption d'un modèle d'additivité simple pour les substances cancérigènes nous permettra de maintenir le risque associé à la présence simultanée de plusieurs de ces substances à un niveau négligeable.

- Compartimentation

  L'approche adoptée par le Menviq pour le contrôle du rejet de toxiques dans le milieu aquatique se fait principalement en fonction de leur impact local. Puisque les critères de qualité doivent être respectés suivant une zone de mélange restreinte, les phénomènes de compartimentation (adsorption sur les particules, sédimentation, volatilisation) et de dégradation ne devraient pas être importants pour la plupart des substances responsables de la toxicité d'un effluent.

Limites des méthodes analytiques

Lorsque la valeur du critère est plus faible que la limite de détection de la méthode analytique, il faut revoir le mode et le lieu d'échantillonnage de la substance. Le bon compartiment (eau, sédiment, biote) doit aussi être choisi. Cette situation peut se produire pour des substances cancérigènes et pour plusieurs substances bioaccumulables.

CONCLUSION

La publication récente de deux documents concernant les critères de qualité de l'eau va permettre d'uniformiser les valeurs de critères qui sont ou qui seront utilisés au Québec pour l'évaluation ou la prévention d'une contamination des milieux aquatiques. Dans une situation donnée, le choix des substances à surveiller, de leurs critères et de l'usage à protéger, des facteurs physiques et chimiques du milieu à considérer relèvent d'un jugement professionnel suivant l'objectif visé par l'utilisateur. Il reste qu'un certain cadre d'application dicté à la base par les fondements scientifiques soutenant les critères, doit être respecté.

Finalement, les critères de qualité spécifiques et les critères de toxicité globale demeurent des outils complémentaires pour le contrôle et l'évaluation des rejets toxiques. Leur application simultanée pour la définition de niveaux d'intervention pour les sources ponctuelles de pollution devrait permettre l'atteinte d'une qualité d'eau adéquate pour le maintien des divers usages de l'eau dans les cours d'eau québécois.

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ONTARIO'S PROVINCIAL WATER QUALITY OBJECTIVES AND THEIR USE IN SETTING SITE-SPECIFIC WATER QUALITY CRITERIA.

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Abstract

Ontario currently has some type of chemical-specific criteria (Objectives, Guidelines or Interim Guidelines) for the protection of aquatic life and recreation for over 150 chemicals. These have a varied history dating from the 1972 USEPA Blue Book to those currently under development. These criteria are based solely either on whole organism laboratory toxicity/bioaccumulation tests, or rarely, aesthetic impairment (taste or odour of water or fish tissue). Implementation of these criteria, as done on a site-specific basis, may take into account the nature of the receiving water body, mixing zones and other considerations. We outline Ontario's current approach to setting water quality criteria and provide examples of implementation.

History

Water quality objectives have been developed over time to meet the needs of the protection of aquatic life. Over the years there have been a number of criteria developed starting with conventional toxicants which were designed primarily to prevent acute toxicity. As toxicological understanding became more sophisticated the clear intent has been, in Ontario at least, to protect all life stages for an indefinite exposure i.e. zero risk to populations.

Ontario has a set of water quality criteria with a diverse history. Those values currently in the "Blue Book" of water management practices in Ontario are derived from a variety of sources. The earliest of these date from the NAS/NAE 1972 Blue Book. Others were taken from IJC documents. Few were developed directly by the province. Recently it has become apparent that Ontario should develop its own criteria and over the past 3-5 years has developed both a list of priority pollutants, and a method to develop chemical-specific criteria for them.

This method has attempted to formalize the process and to remove some of the subjectivity associated with the development of water quality criteria. This has facilitated development by both consultants and Ministry staff.

Protocol

The protocol is documented in *Ontario's Water Quality Objective Development Process (1991)*. The protocol supports existing policy and legislation in the development of chemical specific water quality criteria "to ensure that the surface waters of the province are of a quality which is satisfactory for aquatic life and recreation" (Water Management - goals, policies, objectives and implementation procedures of the Ministry of the Environment 1984). Under this protocol there are two tiers of criteria - objectives where the toxicology and other database meets certain minimum data requirements for both quantity and quality, and guidelines where those conditions are not met (Figure 1).

Development of an Objective

This is based first of all on toxicity data. If sufficient high quality, chronic data covering sensitive life stages of vertebrate, invertebrate and plant life are available then a preliminary Objective is set by dividing the lowest value by a safety factor of 10. Second, bioaccumulation, either reliable bioconcentration factors or log K_{ow}, is considered to reduce the PWQO to prevent accumulation of contaminants in fish at concentrations which may be harmful to fish consumers, both human and wildlife. (Figure 2). This is done by dividing the lowest acceptable residue (i.e. 0.5 mg/kg for mercury set by human health agencies) by the highest reliable BCF and then applying a safety factor of 10. The lower of the toxicity and bioaccumulation values carries forward to the next step - mutagenicity assessment (Figure 3). Finally, other factors are considered such as taste and odour in water, tainting of fish tissues, recreation, and wildlife protection. Sediment quality is addressed by Ontario's Sediment Quality Guidelines. The development of Objectives (and Guidelines) is not influenced by socio-economic, analytic or treatability considerations.

Development of Guidelines

This is a very simple and highly conservative process where the physico-chemical properties and the quantity and quality of toxicity/mutagenicity data determines the final value. Starting with a basic uncertainty factor of either 1000 or, 10000 if log K_{ow} is ≥ 4 (or log BCF ≥ 3) which is reduced as a function of the quantity and quality of the data. A data set which is barely insufficient to produce an Objective receives a final factor of 13.

Site Specific Applications

Under Section 24 of the Ontario Water Resources Act a Certificate of Approval is required for all new or expanded point source discharges. Certificates of Approval specify or limit the quality or volume of effluent discharges. Policy 3 of the "Blue Book" identifies procedures for setting effluent limits.

There are two basic directions for setting effluent limits: 1) to develop limits based on best available technology economically achievable i.e. the MISA Program; or 2) to employ site-specific receiving water assessments which take into account dilution, dispersion, and assimilation capacity of water courses receiving waste water effluents.

PWQO/G's are used in the first case to evaluate instream performance of BATEA limits. In receiving water assessments PWQO/G's are used as targets/design conditions on a case-by-case basis to establish a discharge limit which will protect water quality conditions and receiving water uses.

Design conditions, by which the attainment of PWQO/G's are evaluated, take into account variables such as background flow rates or lake volumes, degraded or pristine receiver water quality conditions and multiple discharges. Very stringent or liberal design conditions can be applied, depending upon the safety factors one wishes to build into the assessment.

An interactive Receiver Assessment Model has been developed jointly with MOE Approvals Branch and Environment Canada's National Water Research Institute. The objective of this model is to expedite head to head negotiations during the review of Certificate of Approval applications. PWQO/Gs are one of the criteria used in this model to evaluate the proposed discharge.

Other approaches under development will provide Regional staff with insight into how often and for how long the proposed discharge would violate PWQO/G's. By coupling estimates of exposure with safety factors built into the PWQO/G's, Approvals staff will be in a better position to evaluate proposals which require deviations in "Blue Book" water management policy. Risk assessments to evaluate elasticity of systems under given water quality upsets will provide additional management tools in reviews of expanded or new discharges.

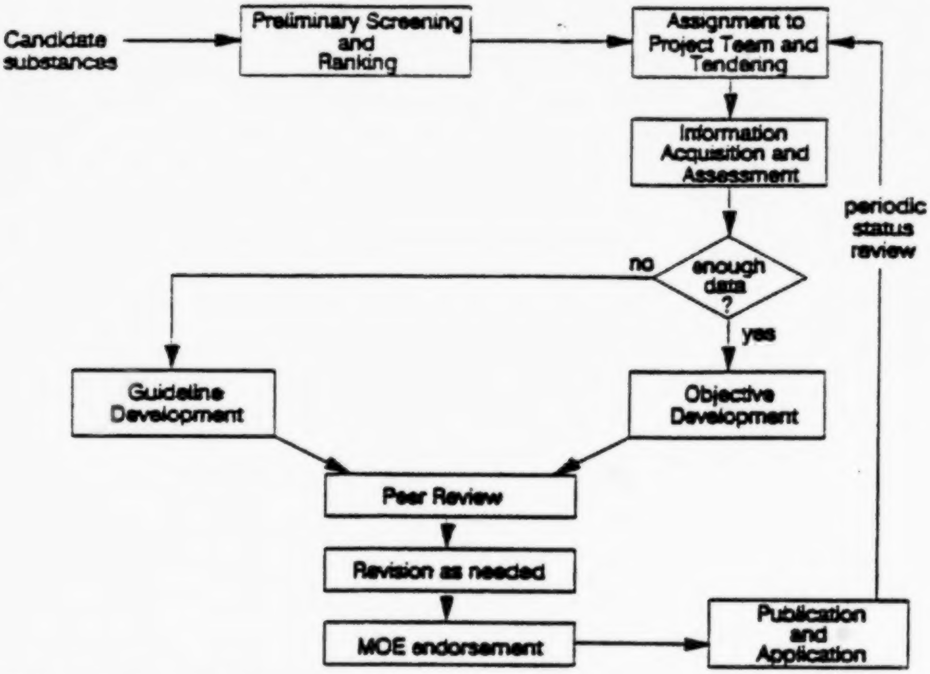
Candidate substances
Preliminary Screening and Ranking
Assignment to Project Team and Tendering
Information Acquisition and Assessment
periodic status review
no
enough data ?
yes
Guideline Development
Objective Development
Peer Review
Revision as needed
MOE endorsement
Publication and Application

Figure 1. The process for developing PWQOs and PWQGs

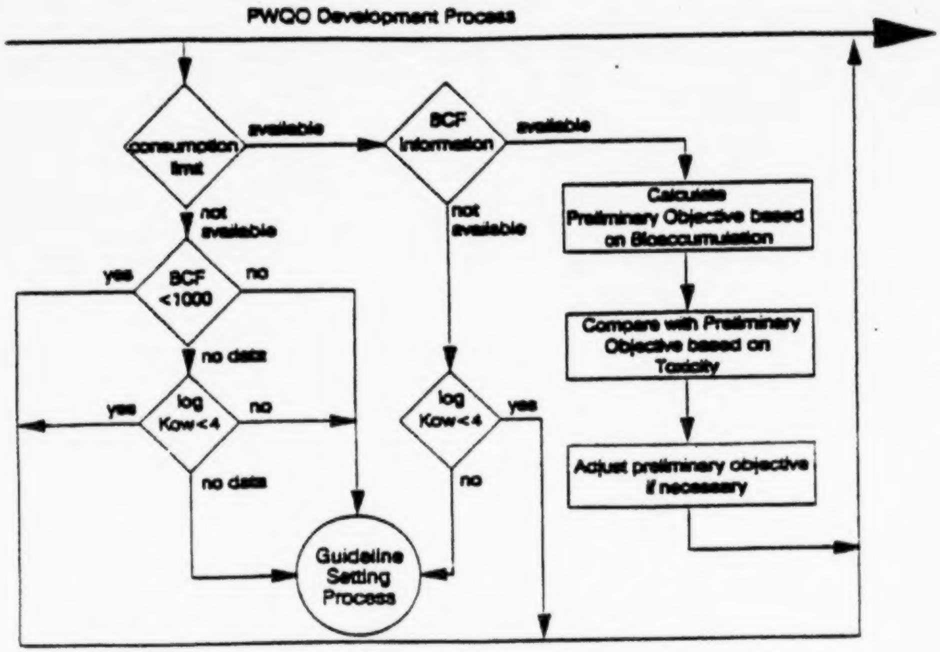
PWQO Development Process
consumption limit
available
not available
BCF Information
available
not available
Calculate Preliminary Objective based on Bioaccumulation
Compare with Preliminary Objective based on Toxicity
Adjust preliminary objective if necessary
yes
BCF <1000
no
no data
yes
log Kow<4
no
no data
log Kow<4
yes
no
Guideline Setting Process

Figure 2. Bioaccumulation assessment for the PWQO development process

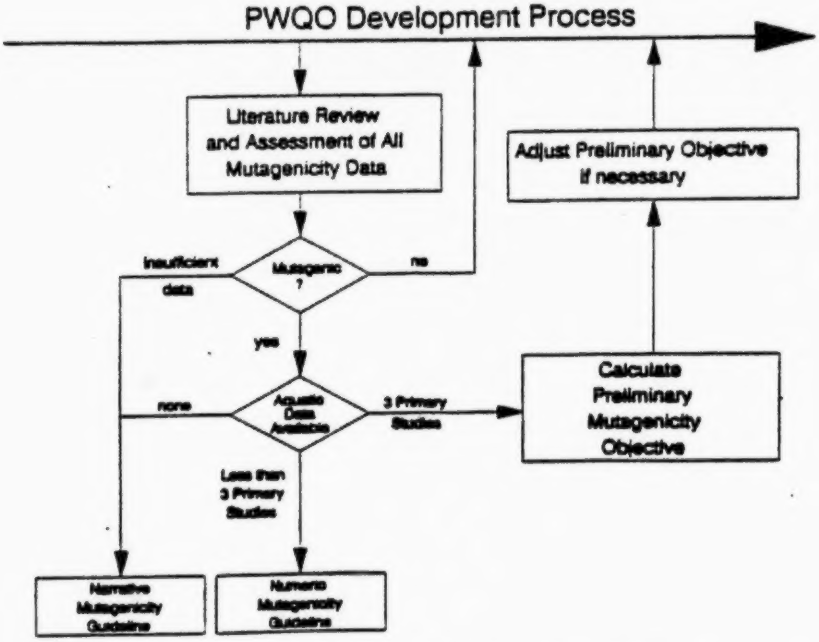
PWQO Development Process
Literature Review and Assessment of All Mutagenicity Data
Adjust Preliminary Objective if necessary
insufficient data
Mutagenic ?
no
yes
none
Aquatic Data Available
3 Primary Studies
Calculate Preliminary Mutagenicity Objective
Less than 3 Primary Studies
Narrative Mutagenicity Guideline
Numeric Mutagenicity Guideline

Figure 3. Mutagenicity Assessment for the PWQO Development Process.

INTERNATIONAL APPROACHES TO ENVIRONMENTAL EFFECTS MONITORING.

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The policies and approaches of several international jurisdictions to aquatic environment effects monitoring of effluents and their effectiveness, and potential applicability to the Canadian situation is reviewed. Specific aspects reviewed included monitoring and reporting, data interpretation, research and development activities and mechanisms for public input.

Environmental effects monitoring (EEM) consists of chemical and biological monitoring of effluent receiving water, sediments and biota which are integrated to provide an indication of overall environmental quality. Contrary to the traditional "technology-based" approach to environmental effects which uses physico-chemical and biological properties of effluent to make inferences about the receiving environment, EEM is a "top down" approach which uses ecological characteristics of the receiving environment (such as aquatic community composition and diversity, population structure, reprodcution and growth characteristics) to evaluate chemical, physical and biological conditions.

Current Programs and future direction of environmental effects monitoring were reviewed for Australia, Finland, Sweden and the USA. Comparison of these jurisdictions reveals that environmental effects monitoring is gaining acceptance and is being increasingly used as a tool for indentifying and assessing the impacts of wastewater discharges to receiving water in a number of countries. Finland still utilizes a predominantly technology based approach, and includes elements of EEM on an ad hoc basis. Australia has developed a national approach to EEM, but has yet to implement it. Sweden and the USA have formulated and implemented national approaches to EEM. The Swedish EEM program has the status of a guideline but is incorporated into permit requirements on a site-specific basis. In the USA a comprehensive EEM program will be a legal requirement of all States by 1993, and a number of States have adopted and/or modified such programs for their own regulatory purposes.

Thre is no one international program which can be used as a "blueprint" for development of a Canadian approach to EEM, however, all programs examined have elements which would be desirable in a Canadian program. The Australian, Swedish and American programs are particularly relevant to the Canadian situation and recommendation for a technically effective and consistent EEM program were made in the following areas of interest: Approach to EEM, administrative framework, public participation, study design and parameter selection, data interpretation and quality assurance. A review of international programs led to the recommendation that the following key elements should be part of a Canadian program: (1) goals must be defined as practical working objectives (i.e zero discharge, no effect, no net effect); (2) develop a federal/provincial administrative process driven top down and ensure consistent national implementation and interpretation; (3) study design and parameter selection must relate directly to EEM objectives and provide national consistency, a minimum of protection, be technically sound and be cost effective; (4) interpretation of data requires national consistency to avoid regional differences in acceptability (ie. water quality objectives, biological vs statistical significance, "scientific judgement"); (5) quality assurance must be part of all EEM elements; and (6) consultation includes community in government/industry negotiations and incorporates education, information transfer, decision making and mechanism for dispute resolution.

A comparison of jurisdictions that had two or more tiers of government (ie. federal/provincial) showed that the responsibility for administering EEM studies is shared between the two levels. The Canadian federal government is committed to development of a cooperative joint federal/provincial process for implementation and administration of the EEM study requirement. Recommendation for such a process were made based on international approaches.

ENVIRONMENT CANADA'S INITIATIVE ON ENVIRONMENTAL EFFECTS MONITORING: TECHNICAL DEVELOPMENT. G.R. Craig, P. Orr, D. Hart and R. Scroggins, Beak Consultants Limited, Brampton, Ontario, and Environment Canada, Ottawa, Ontario.

The underlying principles of study design will be presented based on intended uses of the required physical, chemical, and toxicological effluent data. Minimum level of effort will also be presented in terms of sample replication and sample site selection requirements. The rationales for core and site specific program elements will be explained. The relationship between effluent and sediment characterization, and inference of fisheries and benthic biological effects, will be identified to emphasize the need for sound integrative interpretation of degree and extent of measurable impact.

ALBERTA ENVIRONMENT'S USE OF ECOLOGICAL MONITORING IN EFFLUENT LICENCING. J.B. Kemper, D.O. Trew, L.R. Noton and I. Mackenzie, Alberta Environment, Edmonton, Alberta.

The importance of using environmental effects monitoring data (EEM) for the establishment of effluent standards has become widely accepted. This paper describes selected cases in Alberta which demonstrate the successful application of EEM in the formulation of industrial and municipal license conditions.

Examples discussed will include the regulation of BOD, AOX, and TSS discharges from the expanding pulp mill sector in Alberta, the implementation of phosphorus removal on the City of Calgary's sewage effluent, the pending requirements for disinfection at all major urban centers, and the exclusion of both industrial and municipal effluents from the Highwood River, an important spawning and rearing habitat for the Bow River trout fishery. Issues and specific cases currently under investigation are also reviewed.

Th evolution of monitoring requirements as specified in discharge licenses is described, as are trends in joint industrial/government monitoring programs. The use of real-time monitoring systems in river water quality management is also discussed.

RECENT DEVELOPMENTS IN THE U.S.A. ON THE WATER QUALITY BASED APPROACH AND ECOSYSTEM MONITORING, R. Brandes, U.S. EPA, Washington, D.C.

The traditional approach in the U.S. to setting controls for pollutants discharged by point sources has been a chemical approach. Technology-based national effluent standards as well as water quality standards have, in the past, been expressed in terms of specific pollutants or pollutant parameters such as BOD. Starting in the mid-1980s, the U.S. EPA and States have begun to extensively employ biological methods in point source controls. Whole effluent toxicity in particular has become an integral part of toxics control from point sources. This control component, when linked with Toxicity Reduction Evaluation procedures, has resulted in the reduction of toxicity from many point sources.

EPA currently promotes the policy of "independent applicability" when utilizing the three components of the national water quality program. The components, chemical criteria, whole effluent toxicity criteria, and biological criteria, must be used independently so one leg of the triad is not used to supercede any other component.

New areas in which biological approaches will play a major role are sediment contamination control, use of biocriteria for influencing control decisions, and bioaccumulation control.

SESSION 3D

AQUATIC TOXICOLOGY IN SUPPORT OF THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA)/LA TOXICOLOGIE AQUATIQUE À L'APPUI DE LA LOI CANADIENNE SUR LA PROTECTION DE L'ENVIRONNEMENT (LCPE)

CHAIRPERSON/PRÉSIDENTE
Sheila Jones

DEVELOPMENT OF 10-DAY MARINE/ESTUARINE AMPHIPOD ASSAY FOR SEDIMENT TOXICITY IN SUPPORT OF THE CANADIAN OCEAN DUMPING PROGRAM (CEPA, PART VI).

D.J. McLeay, S.G. Yee, K.G. Doe and L.M. Porebski, McLeay Associates Limited, West Vancouver, British Columbia, Environment Canada, and Aquatic Toxicity Laboratory, North Vancouver, British Columbia, and Environment Canada, Laboratory Division, Dartmouth, Nova Scotia, and Environment Canada, Office of Waste Management, Environment Canada, Ottawa, Ontario.

ABSTRACT

Beginning in 1988, Environment Canada commenced the development of a test method for measuring the acute toxicity of sediment samples, using a number of marine or estuarine sediment-burrowing amphipods common to Canada's coastal waters. The evolution of this toxicity test included five series of inter-laboratory assessments using various candidate test organisms, sediment samples, and a reference toxicant. These studies are reviewed briefly. Additionally, the test method and its applications are summarized.

INTRODUCTION

The Ocean Dumping Control Act has been consolidated into Part VI of the Canadian Environmental Protection Act (CEPA). The Act requires valid ocean dumping permits before dumping of any substance at sea is allowed. Biological testing and assessment can be an integral component of this permit process (Sergy, 1988; Anthony, 1991; Porebski, 1991). In order for Environment Canada to perform its regulatory responsibilities associated with this Act, biological screening tests using marine or estuarine organisms may be required to determine if material is suitable for unconfined open-water disposal, and to perform environmental-effects monitoring at dump sites. Interim contaminant testing guidelines for ocean disposal, associated with Environment Canada's Ocean Dumping Control Program, specify several toxicity tests for use in screening materials. The 10-day assay for sediment toxicity, using one or more species of estuarine or marine amphipods common to Canada's coastal waters, is included on the lists of biological screening tests (Environment Canada, 1990a, 1990b).

In consideration of the above, Environment Canada's Atlantic and Pacific & Yukon regional laboratories commenced inter-laboratory studies in late 1988, using a number of marine or estuarine sediment-burrowing amphipods and various samples of control, reference or test (contaminated) sediments. The objectives of this testing program were twofold: (1) to study several candidate species of amphipods, selecting those suitable for use in acute lethality tests with samples of sediment or other test material; and (2) to evaluate conditions, procedures and biological endpoints appropriate for use in a standard biological test method to be developed to meet Environment Canada's testing requirements in this respect. The past status of this and other biological test methods under development on behalf of Environment Canada has been reported (McLeay et al., 1991a).

Following is a brief summary of the inter-laboratory studies with marine or estuarine amphipods, performed to date by Environment Canada's Pacific & Yukon and Atlantic regional laboratories[1]. Also presented is a list of those species of infaunal amphipods presently recommended for use in Environment Canada's draft biological test method "Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods" (Environment Canada, 1991). Tentative checklists of recommended conditions and procedures for holding and acclimating amphipods, and for testing them in 10-day static assays, are provided. Finally, some of the applications of this biological test method are indicated.

LABORATORY EVALUATION AND DEVELOPMENT OF TEST METHOD

In 1988, Environment Canada's Atlantic and Pacific & Yukon regional laboratories undertook a preliminary (Phase-I) evaluation of the 10-day sediment assay, using only *Rhepoxynius abronius* (McLeay et al., 1989). The effects of holding amphipods in control sediment for periods of up to 81 days, on their 10-day survival and subsequent reburial rates under test conditions (control sediment only), were investigated. Their acute tolerance (96-h LC50/EC50) to the reference toxicant cadmium chloride (seawater-only exposures) was monitored during the prolonged holding period. Although 10-day survival and reburial rates were acceptable ($\geq$90%) in all trials, the reference toxicant tests indicated a declined tolerance of these organisms with extended ($\geq$13 days) periods of holding in the laboratory.

The second (Phase-II) inter-laboratory study (McLeay et al., 1991b) measured and compared the 10-day survival, emergence and reburial rates for a population of *Rhepoxynius abronius* exposed to control[2], reference[3] or test[4] sediment. At each laboratory, survival and reburial rates in control sediment were high (≥90%). No consistent differences in these endpoints were caused by exposure to reference or test sediments, indicating that neither were highly toxic. However, increased rates of emergence from the test sediment were observed.

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[1] Basic test conditions and procedures used for the 10-day static assays were according to Swartz et al. (1985) and ASTM (1990).

A Phase-III study was performed by each laboratory using both Rhepoxynius abronius and Corophium volutator as test organisms (McLeay et al., 1991b). For each species, ten-day survival and subsequent reburial rates were determined for three clean[5] (control or reference) sediments and three test sediments. The clean sediments varied appreciably in grain size, with silt/clay contents of 1%, 82% or 99%. Tests with R. abronius showed highest survival rates (96% and 100%) for control sediment, and lowest survival rates (43% and 78%) for the reference sediment containing 99% fines (silt and clay). R. abronius survival rates for the three test sediments ranged from 72 to 93%. Unlike these findings, survival rates for Corophium volutator were high (93% and 97%) in the extremely fine-grained reference sediment. For both species, reburial and/or survival data[6] showed no consistent response for any of the test sediments examined, although emergence rates indicated an avoidance response to one of the test sediments.

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[2] Control sediment is clean sediment taken from the site where the test organisms were collected, and intended for use in the 10-day test with amphipods. This sediment must contain no test material. It is used to determine the absence of measurable toxicity due to basic test conditions (e.g., temperature, health or handling of test organisms).

[3] Reference sediment is a field-collected sample of sediment, taken from a site thought to be relatively free of contaminants (i.e., "clean" sediment), and intended for use in the 10-day test with amphipods. It is often collected from a site within the general vicinity of a test sediment, and is frequently selected for biological testing because of its geochemical similarity (e.g., particle size, compactness, total organic content) to the test sediment(s).

[4] Test sediment is a field-collected sample of solid-phase sediment, taken from a site thought to be contaminated with one or more chemicals, and intended for use in the 10-day test with amphipods. In this study, the sample of test sediment was collected from the vicinity of a B.C. coastal pulp mill discharging bleached kraft mill effluent.

[5] Clean sediment is sediment (e.g., control or reference sediment) that does not contain concentrations of contaminants which cause discernible distress to the test organisms or reduce their survival in 10-day assays.

A fourth inter-laboratory assessment[7], using six species of marine or estuarine infaunal amphipods[8] common to Canada's coastal waters, was undertaken during late 1990 and early 1991 (Paine and McPherson, 1991a). The objectives of this (Phase-IV) study were to determine and compare the relative sensitivity of each of the six test species to four test sediments, two reference sediments (fine-grained and coarse-grained), and control sediments (one for each species). Results for this study showed good inter-laboratory agreement. For the four test sediments examined, each of the six species of amphipods used in these assays distinguished the same two sediments as clearly toxic, and the remaining two as marginally or not toxic. Percentage survival at 10 days was the most useful biological endpoint; little if any additional information was obtained using the other two endpoints (% emergence, % of survivors that did not rebury in control sediment within 1 h following test completion). _E_. _washingtonianus_ and _R_. _abronius_ were most sensitive to the test sediments; _C_. _volutator_ and _E_. _estuarius_ were least sensitive. Two of the six species studied (_E_. _washingtonianus_ and _A_. _virginiana_) showed unacceptably low (<90%) 10-day survival rates in control sediment. Depending on species, grain-size effects were minimal or not evident. This study also demonstrated that, if care is taken, amphipods can be shipped across the country without influencing the test results.

Additional studies were performed by Environment Canada's Atlantic regional laboratory in early 1991 to assess the laboratory hardiness and worth of _Amphiporeia_ _virginiana_ as a test organism (Doe, 1991). Two populations of field-collected specimens were tested for 10-day survival rates in control sediment only. Animals were acclimated and tested at temperatures of 10 or 15 C (Series 1); and at 5, 10 or 15 C (Series 2). Results from these studies showed that acceptable (≥90%) 10-day survival rates could be obtained at 5 or 10 C, but not at 15 C. Seasonally-cold (2 to 4 C) seawater temperatures at collection sites likely contributed to these findings.

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[6] Since _C_. _volutator_ do not rebury readily in control sediment within 1 hour, this biological endpoint cannot be determined for this species.

[7] Participating laboratories included Environment Canada's Atlantic and Pacific & Yukon regional laboratories, and EVS Consultants Ltd.

[8] Test organisms were _Rhepoxynius_ _abronius_, _Foxiphalus_ _xiximeus_, _Eohaustorius_ _estuarius_, _Eohaustorius_ _washingtonianus_, _Corophium_ _volutator_, and _Amphiporeia_ _virginiana_.

A fifth inter-laboratory[9] appraisal of the 10-day test for sediment toxicity, using multiple species of marine or estuarine amphipods, was conducted during June 1991 (Paine and McPherson, 1991b). For this study, seven species of infaunal amphipods[10] were collected and examined for their laboratory adaptability and sensitivity to each of three test sediments. Biological endpoints (% survival and % emergence at 10 days; % reburial of survivors in control sediment at test end) were determined for each species in these sediments as well as in a fine-grained (79% silt/clay) reference sediment and respective control sediments. Assays with Amphiporeia virginiana were performed at both 10 and 15 C; all other species were tested at 15 C only. Unlike the previous (Phase-IV) inter-laboratory study, none of the three test sediments used in this study were highly toxic to any of the species of amphipods examined. Once again, ten-day survival rates in control sediment were unacceptably low (<90%) for Amphiporeia virginiana (both temperatures) and Eohaustorius washingtonianus, but acceptable for all other species studied including Leptocheirus pinguis. As in the Phase-IV study, % survival at 10 days was the most useful biological endpoint, with little if any additional information provided by the secondary endpoints (% emergence, % of survivors not reburying in control sediment at test end).

RECOMMENDED TEST SPECIES

Recent attempts have been made to identify suitable Canadian collection sites[11], and to collect the following species of amphipods from Canadian coastal waters, in order to evaluate their worth as candidate test organisms in 10-day sediment assays:

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[9] Environment Canada's Atlantic and Pacific & Yukon regional laboratories.

[10] Test organisms included Rhepoxynius abronius, Foxiphalus xiximeus, Eohaustorius estuarius, Eohaustorius washingtonianus, Corophium volutator, Amphiporeia virginiana, and Leptocheirus pinguis.

[11] Specimen collections held by the National Museum of Natural Sciences (Ottawa, Ontario) were examined by Dr. E.L. Bousfield, together with historical records available to him (e.g., Bousfield, 1990a,b; Bousfield, 1991; Bousfield and Laubitz, 1972). Based on these and other distributional and life-history information, initial recommendations were made for prospective test species.

Pacific Coast	**Atlantic Coast**
Monoculodes spinipes	Rhepoxynius hudsoni
Grandifoxus grandis	Phoxocephalus holbolli
	Ampelisca abdita
	Ampelisca vadorum
	Amphiporeia lawrenciana
	Pontoporeia femorata

The absence or relative scarcity of these species at the Canadian collection sites investigated, prevented their inclusion in the present test program.

Based on the results of the laboratory studies mentioned previously (see section "Laboratory Evaluation and Development of Test Method"), as well as those for other 10-day assays performed with the candidate species of infaunal amphipods under consideration by Environment Canada, the following species of marine or estuarine amphipods are presently recommended for use in 10-day static sediment assays (Environment Canada, 1991):

Recommended Pacific Species	**Recommended Atlantic Species**
Rhepoxynius abronius	Corophium volutator
Foxiphalus xiximeus	Leptocheirus pinguis
Eohaustorius estuarius	

Additional studies with Amphiporeia virginiana and Eohaustorius washingtonianus are required to demonstrate that satisfactory survival rates in control sediment can be achieved for these species, before they can be recommended by Environment Canada as standard test organisms.

RECOMMENDED CONDITIONS AND PROCEDURES FOR ACCLIMATING AND TESTING AMPHIPODS

Environment Canada's draft biological test method (Environment Canada, 1991) provides details regarding conditions and procedures for holding and acclimating amphipods to be used in 10-day assays, as well as those necessary to perform the test in a standardized manner. The test methods of Swartz et al. (1985) and ASTM (1990) formed the basis for Environment Canada's (1991) acute test for sediment toxicity using marine or estuarine amphipods common to Canadian coastal waters. Acclimation and test procedures recommended in Environment Canada (1991) are reproduced here (see Tables 1 and 2). Since this biological test method is not yet finalized and approved by Environment Canada, these procedures and conditions are subject to change.

Table 1 Checklist of Recommended Conditions and Procedures for Holding and Acclimating Amphipods[12]

Source of amphipods:	- Collected subtidally or intertidally from clean sediment
Life stage:	- juveniles or young adults, 3 - 10 mm length (depending on species)
Sorting:	- Sieve through 1.0-mm screen to confirm species and select appropriate size; use seawater within 2 C and 2 ppt salinity of the seawater in transport container
Holding sediment:	- Control sediment, 2-4 cm in depth, previously sieved through 0.5-mm mesh
Holding seawater:	- Reconstituted or clean natural seawater
Acclimation conditions:	- Salinity of seawater same as that for overlying seawater in test; temperature normally 15 ± 2 C; dissolved oxygen ≥90% of air saturation; temperature, salinity, and dissolved oxygen measured and recorded daily
Lighting:	- Constant overhead illumination, ≥100 lux at surface of sediment in holding/acclimation container(s)
Feeding:	- None
Duration of acclimation:	- 3 to 10 days
Health criteria:	- Select amphipods able to bury quickly in control sediment; remove inactive amphipods that have emerged from sediment or do not bury; discard population if ≥5% dead or emerged and inactive during 48-h period preceding test

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[12] From Environment Canada's (1991) draft biological test method - subject to change.

Table 2 Checklist of Recommended Test Conditions and Procedures[13]

UNIVERSAL

Test type:	-	Static, 10-day duration
Test vessel:	-	1-L glass beaker or jar, internal diameter approximately 10 cm
Control sediment:	-	Clean sediment, usually from the site where test organisms were collected; sieved through 0.5-mm screen using test water; depth in test vessels, 2 cm
Test water:	-	Clean seawater, natural or reconstituted; salinity, frequently 28 ± 2 ppt unless estuarine sediments are tested; temperature, 15 ± 2 C; dissolved oxygen, ≥90% saturation; added to 950-mL mark on test vessel
Aeration:	-	Aerate water in each test vessel overnight prior to start of test, and throughout test
Lighting:	-	Constant overhead illumination, ≥100 lux at surface of sediment in test vessels
Amphipods:	-	Juveniles or young adults, 3 - 10 mm length (depending on species); 20 per test vessel; replace animals not burying in sediment within initial 10 min
Number of replicates:	-	Five (20 individuals/replicate) for control sediment(s) and each concentration of reference toxicant; normally five for each test concentration
Feeding:	-	None
Observations:	-	Daily for numbers of amphipods emerging from sediment; at termination of test for death, numbers emerged, and, where measured, numbers not reburying in control sediment within 1 h

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[13] From Environment Canada's (1991) draft biological test method - subject to change.

UNIVERSAL (cont'd.)

Measurements: - Temperature at least daily; pH, salinity and DO of water overlying test and control sediments at least at test start and end; interstitial salinity; daily or less frequent for sediment Eh and/or pH

Endpoints: - As specified and/or dependent upon test objectives and test material; at least 10-day % mortality for single-concentration test; 10-day EC50s and/or 10-day LC50 for multi-concentration tests

Reference toxicant test: - 10-day LC50 with mixture of cadmium or fluoranthene in control or other clean sediment recommended; seawater-only exposures can also be used

Test validity: - Invalid if overall mortalities in control sediment >10%, or if >20% of amphipods in one or more control vessels die during test

FIELD-COLLECTED SEDIMENT

Transport and storage: - Transport and store in darkness at 4 ± 3 C, do not freeze; test within two weeks

Reference sediment: - One or more samples required; taken from sites presumed to be clean but in the general vicinity of those where test sediments are collected; ideally, particle size and organic content within the range of test sediments

Sediment characterization: - Particle size distribution, organic carbon content, % moisture; salinity of interstitial water; might measure temperature, pH, Eh, biochemical oxygen demand, chemical oxygen demand, and/or specific contaminants in solid phase or interstitial water

Preparation of sediment: - Sediment normally not wet-sieved; special circumstances (e.g., interstitial water oxygen-deficient or low in salinity) might require wet-sieving using test water

Test water: - Clean seawater, natural or reconstituted

SLUDGE OR OTHER SOLID WASTE

Transport and storage: - As for field-collected sediment

Sediment characterization: - As for field-collected sediment

Preparation of sediment: - Remove water as necessary, by centrifuging at speed; wet-sieving with test water necessary if interstitial water low in salinity or oxygen-deficient; other circumstances or test objectives (e.g., selection of fraction with specific particle size) might also require wet-sieving; if sample known or suspected to be toxic, might require preparation of a range of test concentrations (i.e., mixtures using control sediment)

Test water: - Clean seawater, natural or reconstituted

CHEMICAL

Characterization: - Information required concerning water solubility, vapour pressure, stability, biodegradability, purity, etc.

Solvent: - Test water is the preferred solvent; if an organic solvent used, must include a solvent control

Preparation of test mixtures: - Procedure dependent upon test design and objectives; might include one or more chemical concentrations mixed in control or test sediment, or specific chemical concentrations added to the test water overlying control sediment; chemical-sediment mixtures may be prepared manually or by mechanical agitation as slurries or dry mixtures

Concentration: - Desirable to measure at beginning and end of exposure, in high, medium and low strengths

Test and dilution water: - Reconstituted seawater if a high degree of standardization is required; otherwise clean, natural seawater is acceptable

APPLICATIONS OF TEN-DAY AMPHIPOD ASSAY

This biological test method can be applied in the laboratory to provide information regarding a variety of questions associated with the short-term adverse effects of contaminants in sediment. Depending on the species of amphipods selected, the test can be suitable for toxicity assessments related to the estuarine or marine environment. The method can be used for regulatory, compliance monitoring, toxicity reduction evaluations, or other investigative purposes. It can also be incorporated in guidelines or objectives associated with the development or validation of sediment quality criteria.

Regarding Environment Canada's ocean dumping control program, this acute test for sediment toxicity can be used to screen materials and assess their suitability for open-water disposal, or to appraise the impact of ocean dumping on marine environmental quality at designated dump sites. Other applications related to CEPA include the evaluation of new or in-use chemicals, chemical products or formulations on sediment quality, when specific concentrations are mixed with sediment or added to seawater overlying sediment. The test can be used to examine the acute toxicity of samples of soil from industrial sites, industrial or municipal sludge, or marine or estuarine sediment from the vicinity of industrial or municipal discharges. For instance, the test can be included as part of field surveys concerned with the spatial and/or temporal distribution of contaminants in marine or estuarine sediments.

Questions related to causal toxicity of sediment, or to processes of degradation or recovery of contaminated benthic ecosystems, can be addressed using the test. This procedure can also be useful for testing the effects of various geochemical characteristics of sediments on marine or estuarine amphipods. When combined with other appropriate sediment bioassays, chemical analyses and field surveys of benthic communities, this biological test method can provide an integral component for meaningful appraisals of environmental quality.

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EVALUATION OF FOUR FRESHWATER BENTHIC INVERTEBRATES FOR ASSESSMENT OF CONTAMINATED SEDIMENTS AND THEIR RELEVANCE TO CEPA.

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EXTENDED ABSTRACT

The Canadian Environmental Protection Act (CEPA) gives the Minister of the Environment the authority to request from importers, manufacturers, transporters and/or distributors of new substances or substances already in existence in Canadian commerce, information on the immediate or long-term harmful effects of these substances in the environment. In addition, under CEPA, there is a priority list of substances that are suspected of having adverse environmental effects due to their persistence, potential for bioaccumulation, widespread use and/or toxicity. Data relevant to understanding the impact of these substances on the structure and function of populations within ecosystems and their ability to concentrate in living organisms are required and risk assessments of these toxicants must be completed by 1994.

Sediments are an integral part of the aquatic environment providing habitat, food and refuge for many aquatic biota. Many toxic contaminants, particularly those on the CEPA priority list, occur in only trace amounts in the water column but may accumulate in sediments to elevated levels. These levels may have deleterious effects on aquatic biota living in close association with the sediment either through direct ingestion of contaminated particles or through passive diffusion from the pore water. Unfortunately, little information on the toxicity of contaminated sediments to aquatic biota is available and few methodologies to determine sediment toxicity have been standardized.

Research is presently being conducted at the National Water Research Institute (Environment Canada, Burlington, Ontario) to gain experience and develop methodologies for whole sediment bioassays using several species of freshwater benthic invertebrates i.e., the amphipod, *Hyalella azteca*, the tubificid worm, *Tubifex tubifex*, the chironomid, *Chironomus riparius* and the mayfly, *Hexagenia limbata/rigida*. The test methods allow an assessment of the chronic toxicity of contaminants in sediments on survival, growth and/or reproduction of benthic invertebrates and will be incorporated into Environment Canada's guidance documents (e.g., Environment Canada 1990a) which describe "core" aquatic toxicity tests for ensuring consistency and quality in environmental protection. The four species are also being assessed for their ability to discriminate between samples and their sensitivity.

The necessity for standardized laboratory procedures for sediment-based biological toxicity tests are 1. To determine the toxicity of new chemicals added to sediment (i.e., spiked-sediment) 2. To compare the toxicities of different chemicals in sediment. 3. To compare sensitivities of different species. 4. To identify areas for remedial action. 5. To determine acceptability of sediments for open-water disposal. 6. For the derivation of sediment quality criteria. 7. To determine bioaccumulation potential. 8. To define effects of environmental factors on toxicity (e.g., temp.,pH) 9. Provide data on chemical mixtures in field sediments.

Laboratory culture of the four benthic invertebrates was initiated in early 1991 using all available information from published protocols and these species are being used to assess the toxicity of contaminated sediments in areas of concern in the Great Lakes and for the development of sediment quality criteria. During this time, several modifications and/or concerns from existing methodologies have been necessary in order obtain useful toxicological information.

For example, it is well-known that the collection of field sediments and their preparation for laboratory sediment toxicity tests disrupts the natural state of the sediment and may alter the bioavailability and chemical equilibrium of associated contaminants (ASTM 1991). For example, resuspension of the sediment during manipulation may allow adsorbed toxicants to re-enter the dissolved phase and increase toxicity to aquatic biota from the pore water. As a result, most protocols for sediment bioassays suggest that field-collected sediments not be sieved and that only large objects (e.g., rocks, twigs, etc.) or obvious endemic organisms (e.g., leeches, dragonflies, etc.) be removed with tweezers. This is often impossible to do during freshwater sediment bioassays utilizing organisms such as tubificids and/or chironomids where similar endemic species are present in field-collected sediments. For example, we have run toxicity tests with sediment collected from Long Point, Lake Erie, which contains an endemic chironomid very similar to *C. riparius*. Even sieving through 250 µm mesh did not remove this species and the presence of morphologically similar organisms makes interpretation of toxicity endpoints such as survival, impossible.

We have been experimenting in our laboratories with ways to remove endemic species other than sieving. For example, we have compared sieving (through two mesh sizes, 500 and 250 µm) to autoclaving, freezing and gamma-irradiation of sediment using the tubificid bioassay and found the following. Sieving through 500 µm mesh allowed more competitors and predators to remain in sediment resulting in lower counts for total young production compared to sieving through 250 µm mesh. No treatment of sediment (i.e., no seiving) resulted in few, if any, young. Autoclaving appears to have a detrimental effect on the production of young. Reasons for this may include the removal of bacteria and other microorganisms which are a food source for tubificids on the sediment ingested. Freezing and irradiation of sediment allowed the highest production of young. However, freezing of sediments which contain toxicants, especially metals (Malueg *et al.* 1986), is not recommended due to reported changes in the dissolved state of many of these chemicals. Gamma-irradiation resulted in the highest production of young and may be explained as follows: irradiation was set only to kill organisms at a higher trophic level than bacteria, thus allowing the source of food for tubificids to remain while removing all competing species and/or predators. Based on this information, we have been irradiating our sediment prior to running toxicity tests.

Other factors which have been found to affect the results of bioassays include sexual dimorphism in the chironomid bioassay e.g., female weight in the 4th instar can exceed male weight two-fold but females cannot be distinguished from males until after emergence. The result of this dimorphism in a worst-case scenario could be a pronouncment of toxicity in sediments where a predominace of males were unknownly added to bioassay containers. In addition, we have found that wet weight in the determination of growth for all bioassay species is very variable i.e., wet weight constantly decreases over time as the 'blotted' animals sit on a microbalance. Therefore, we have been experimenting with the use of an image analyzer and are in the process of correlating wet weight to length as a way to quantify growth.

A final component of sediment bioassay procedures to be discussed is the concept of QA/QC in laboratory sediment bioassays (Environment Canada 1990b). Any laboratory conducting sediment bioassays on a routine basis is faced with decisions about control or reference sediments and the use of reference toxicants. The field of sediment toxicity testing is very new relative to water-borne toxicity testing and considerable work is required before the most effective QA/QC approaches are identified.

The response determined in sediment toxicity tests must be compared with the response in a control sediment i.e., a negative control or reference sediment for the following reasons: a) To measure the acceptability of the test with regard the i) health of the test organisms and the ii) suitability of the overlying water, test conditions and handling procedures and b) as a basis for interpreting data obtained from tests in other sediments.

It is often difficult in areas of contaminated sediment to find clean sediment that is physically similar and nutritionally acceptable for the test organisms to serve as a control or reference sediment. This usually means that a reference sediment must be collected from other aquatic areas known to be "clean" and such sediment may vary seasonally in terms of its organic content, endemic species, bacterial presence, etc., and thus not be a consistent source. This problem has been overcome to some extent in EPA laboratories in the United States doing sediment toxicity tests through the use of a standard terrestrial soil collected form a site known to be free of any contaminants. In our laboratory, we are in the process of searching for a local source of soil which closely matches the type of soil used in North American EPA laboratories i.e., Florissant silt loam soil with % H_2O = 39, total organic carbon = 1%, inorganic carbon = <0.01% and particle size (8% sand, 66% silt, 26% clay).

As a final consideration of QA/QC, tests with reference toxicants are a necessity and are used to control intralaboratory test precision (Environment Canada 1990). These tests can be employed using either spiked sediment or short-term (i.e., 96-h) water-only toxicity tests to generate LC50 or EC50 values. There are advantages and disadvantages to using either bioassay systems but spiking of sediments, particularly with metals, appears to have greater disadvantages. For example, the spiking of a sediment with a toxicant is difficult in terms of consistency of distribution of the toxicant within the substrate and variations in the equilibrium partitioning of the toxicant between the dissolved and adsorbed states from bioassay to bioassay. On the other hand, the exposure of organisms which are thigmotactic, i.e., require contact with a substrate for normal functioning, can result in greater toxicity in treatments and problems with control mortality. Our laboratory is presently utilizing both spiked sediment exposures and acute, water-only exposures for reference toxicants in order to evaluate the best scenario for QA/QC.

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ASSESSMENT OF NEW CHEMICALS FOR THE CANADIAN ENVIRONMENTAL PROTECTION ACT. R.A.F. Matheson, Environment Canada, Commercial Chemicals Branch, Ottawa, Ontario.

The Canadian Environmental Protection Act (CEPA) requires that all new substances be assessed for "toxicity" as defined by the legislation. Proponents of new substances for the Canadian market are required to provide detailed information and test data prior to the commencement of any manufacture or import. Assessment for potential adverse environmental and human health effects is a joint resposibility of Environment Canada and Health and Welfare Canada. The Departments must complete the assessment within a specified time period and, if the substance is suspected to be toxic, implement the appropriate controls.

Regulations are under development for the purpose of identifying data requirements and acceptable test methodologies for substance categories (e.g. chemicals, polymers). Environmental fate and effects will be evaluated on the basis of intrinsic physical/chemical properties, environmental stability and compartmentalization and most importantly, aquatic toxicity.

TOXICITY ASSESSMENT OF BIOTECHNOLOGY PRODUCTS UNDER THE CANADIAN ENVIRONMENTAL PROTECTION ACT (CEPA). T. Edge, T. McIntyre, Environment Canada, Commercial Chemicals Branch, Ottawa, Ontario.

New Substances Notification Regulations for biotechnology products are being developed under the Canadian Environmental Protection Act. The regulations will require proponents to notify Environment Canada prior to the importations, manufacture or environmental release of biotechnology products in Canada. The regulations will apply to microbial products, both naturally occurring and genetically modified, that are not regulated by other federal legislation. The regulations will specifiy what environmental data must be provided by proponents in order to assess whether their biotechnology product is toxic. The information requirements for assessing toxicity under the regulations will be risk based. Protocols on environmental effects testing for the aquatic environment are under development.

INTERNATIONAL HARMONIZATION OF ENVIRONMENTAL TESTING PROCEDURES. G.R. Biddinger, S.J. Pauwels and M.L. Hinman, Exxon Biomedical Sciences, Incorporated, East Millstone, New Jersey.

Developing standardized methods is an integral step in creating uniform and acceptable data for regulatory purposes. Unfortunately, as each nation or regulatory body attempts to acheive standardization, they often find themselves out of step with each other. Differences in protocols can be the basis for the rejection of data by other regulatory bodies even though the results would not be substantially different. As every dollar spent for environmental protection is critical, it is an important goal that redundant testing be eliminated whenever possible. Therefore, harmonization of test methods is an important aspect of standardization of data collection. The purpose of this paper will be to review the intricacies of standardization of test methods. The major standarization organizations will be identified and their relevance to environmental regulations discussed. To highlight how test methods can differ, the recently released guidelines from Environment Canada for acute toxicity testing with fish and Daphnids will be compared to U.S. and OECD guidelines. The relevance of these differences will be discussed and areas for harmonization identified.

OCEAN DUMPING: SEDIMENT QUALITY GUIDELINES IN A REGULATORY FRAMEWORK/IMMERSION DE DÉCHETS EN MER: LES RECOMMANDATIONS SUR LA QUALITÉ DES SÉDIMENTS À L'INTÉRIEUR D'UN CADRE LÉGISLATIF

CHAIRPERSONS/PRÉSIDENTES
Linda Porebski and Sherri Smith

A PROTOCOL FOR THE DERIVATION AND USE OF CANADIAN SEDIMENT QUALITY GUIDELINES.

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Sediment quality has emerged as a dominant issue in considerations of both freshwater and marine environmental quality in Canada. For this reason, the Canadian Council of Ministers of the Environment (CCME) has identified the development of sediment quality guidelines for freshwater and marine ecosystems as one of its highest priorities. These guidelines are required to provide resource and environmental managers with the scientific and technical information needed to assess ambient environmental quality, to regulate contaminant sources, and to assess the quality of dredged materials. In addition, these guidelines are needed to support the establishment of site specific sediment quality objectives and to define cleanup goals when remedial measures are required.

The recommended protocol is designed to fulfill the immediate need for sediment qaulity guidelines while making use of the best information currently available to assess the effects of sediment-sorbed contaminants. A formalized procedure is recommended that relies, preferentially, on the results of spiked-sediment bioassays. Alternate procedures are recommended for the derivation of interm guidelines when insufficient toxicity data exist to support the derivation of guidelines. Guidelines from other jurisdictions and information on background levels under certain circumstances.

A two-level approach is recommended to provide the requisite tools for environmental decision-making processes. A framework is also provided for the use of these guidelines.

EVALUATION OF DREDGED MATERIAL FOR OPEN WATER DISPOSAL: THE U.S. ARMY CORPS OF ENGINEERS' EFFECTS-BASED TESTING PROGRAM. T. Dillon, Waterways Experiment Station, Environmental Laboratory, Vicksburg, Mississippi.

The U.S. Army Corps of Engineers has statutory authority to regulate disposal of dredged material in U.S. waters under 404(b)(1) of the Clean Water Act (PL 92-500) and in the oceans under 103 of the Marine Protection, Research and Sanctuaries Act (PL 92-532). The Corps has developed, in consultation with the U.S. Environmental Protection Agency, an effects-based tiered testing protocol for the ocean disposal of dredged material. This approach utilizes acute and chronic bioassays to assess impacts associated with exposure to bedded or suspended sediments. In selecting the effects-based approach, both agencies recognize that dredged material is a very complex substance which may contain a variety of contaminants, both known and unknown. Contaminant bioavailability varies considerably and is significantly affected by the physico-chemical characteristics of different sediment types. Once accumulated by aquatic biota these contaminants ccan interact in poorly understood or unknown ways. For these reasons, chemical concentrations alone cannot be used to assess the potential impact of dredged material. An overview of the effects-based tiered testing protocol used by the Corps of Engineers to evaluate dredged material for ocean disposal is presented.

PROVINCIAL SEDIMENT QUALITY GUIDELINES. R. Jaagumagi, Ontario Ministry of the Environment, Water Resources Branch, Toronto, Ontario.

Ontario has recently developed a set of Provincial Sediment Quality Guidelines (PSQGs) to replace the existing Open Water Disposal Guidelines. The guidelines use three different levels of biological effects: a No-Effect Level based on protection against biomagnification through the food chain, a Lowest Effect Level based on protection of benthic organisms, and a Severe Effect Level based on pronounced adverse effects on benthic organisms. The new set of guidelines are based on two methods of calculation: the Equilibrium Partitioning Approach which uses Provincial Water Quality Objectives/Guidelines, and a co-occurrence analysis based on sediment contaminant concentrations and benthic invertebrate distributions (Screening Level Concentration method). An implementation procedure has also been developed for these guidelines for application to dredging activities, lakefilling, sediment assessment studies and spills clean-up. The guidelines can also be used to deriving clean-up criteria in areas of historical sediment contamination. A procedure that takes into account the "background" levels of contaminants has also been incorporated into the application methodology.

BIOLOGICAL SEDIMENT CRITERIA. T.B. Reynoldson and K.E. Day, Environment Canada, National Water Research Institute, Burlington, Ontario.

Of the 42 designated Areas of Concern in the Great Lakes, all but a few have contaminated sediments. There has been considerable effort over the past few years, particularly by the USEPA and the Ontario Ministry of the Environment, to develop numerical sediment criteria based on chemical concentrations of toxicants in sediment using various methodologies. These techniques require large data bases and relate observed biological effects to distribution statistics for chemicals. The former is dependent on existing water quality criteria which is not always available, the latter makes the assumption that single chemicals are producing the observed biological responses. An alternative to chemical determinations is the development of biological guidelines to determine the need for remediation. In this paper it is proposed that establishing target benthic communities and bioassay endpoints is a much more logical approach. An example of how such guidelines may work is illustrated by the data from the Detroit River. Using benthic invertebrate distribution data from a series of clean sites, it was possible to develop a predictive model for the type of organisms expected at the new sites. This model was used to predict the species (guidelines) at a second set of sites. Stations upstream of effluents met the guidelines and those downstream did not. This suggests that a workable set of biological targets can readily be identified and used as a practical management tool.

ENVIRONMENT CANADA'S OCEAN DUMPING CONTROL PROGRAM. L. Porebski and J. Karau, Environment Canada, Ottawa, Ontario.

The Government of Canada has long accepted its primary responsibility for the management and protection of marine waters from the effects of substances disposed at sea. In 1975, the Ocean Dumping Control Act (ODCA) was passed and in 1988 the Act and Regulations were incorporated into Part VI of the Canadian Environmental Protection Act (CEPA). Environment Canada administers CEPA Part VI and the Ocean Dumping Regulations.

At the present time, the Ocean Dumping Control Program issues permits for the disposal of certain types of material at specific dump sites. Most of the material is dredge spoils from harbours and navigational channels. All materials to be disposed of are required to meet criteria as set forth in CEPA Part VI, Ocean Dumping Regulations and Environmental Canada policies and guidelines.

Over the next few years, the regulatory regime of the Ocean Dumping Control Program will be amended to incorporte a revised fee structure, revised application forms, and requirements for waste audits. Assessment of the suitability of substances for ocean disposal will incorporate biological, as well as chemical and physical assessment procedures. Marine sediment quality guidelines (no effects levels) and rejection limits are being developed and will be incorporated into the regulatory framework. Improved compliance and effects monitoring activities will be undertaken to ensure that the control strategies being implemented are effective in maintaining marine environmental quality.

The components of the Ocean Dumping Control Program will be described, including the tiered testing strategy that will be used in the assessment process.

SPECIAL INTERNATIONAL SESSION ON CONTAMINANTS IN THE ARCTIC/SÉANCE SPÉCIALE INTERNATIONALE SUR LES CONTAMINANTS DANS L'ARCTIQUE

CHAIRPERSON/PRÉSIDENT

Derek Muir

TEMPERATURE AND THE GLOBAL DISTRIBUTION OF LOW VOLATILE ORGANIC COMPOUNDS.

F. Wania and D. Mackay, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario.

ORGANOCHLORINE CONCENTRATION LEVELS IN THE ENVIRONMENT

In the past decade a number of investigations has been performed to establish organochlorine concentrations in Arctic air, water, snow, plants and animals. These data reveal some interesting features as follows.

Example 1: Arctic air concentrations

Table 1 compares the concentrations of organochlorinated compounds in the Arctic with measurements in more temperate regions.

Table 1: Mean air concentrations of organochlorines measured in the Arctic in pg/m^3 (modified from Bidleman et al.,1990)

	HCB	HCHs	Chlordane	DDTs	PCBs	PCCs	Ref.
Svalbard Summer 1984	154	535	(2.8)^a	-	21^c	-	(Pacyna et al., 1988)
Bjørnøya Summer 1984	57	173	(1.3)^a	-	8^c	-	(Pacyna et al., 1988)
Hopen 1982/83	100-250	255-1775	(1.0-2.0)^a	-	10-70^d	-	(Oehme et al., 1984)
Jan Mayen Island 1982/1983	50-200	400-1650	(0.5-2.0)^a	-	50-300^d	-	(Oehme et al., 1984)
Ice Island June 1987	109	240	(5.1)^b	<1	14^d	-	(Hargrave et al., 1989)
1986/87	171	488	8.0	2.7	6.4	40	(Patton et al., 1989)
Alert Feb./April 1988	153	189	3.9	1.4	49	17	(Patton et al., 1991)
Bering/Chukchi Sea July/Aug. 1987	210	319	8.1	-	-	38	(Hinckley et al.,1991)
Egbert, Ontario 1988/1989	-	205	39	89	188	26	(Hoff et al., 1991)
Southern Sweden 1983/1985	64	489	8.4	7.2	165	25	(Bidleman et al., 1987)
Southern Germany Winter 1987	114	286	-	5	670	-	(Ballschmiter et al., 1991)

^{a} cis-chlordane only, ^{b} cis-chlordane and trans-chlordane only, ^{c} pentachlorobiphenyl only, ^{d} quantified as Aroclor mixtures

Hexachlorocylcohexanes (HCHs) and hexachlorobenzene (HCB) and - if determined - polychlorinated camphenes (PCCs) seem to be the most abundant organochlorines in Arctic air, and their concentrations lie within the range measured in many temperate locations. Higher concentrations of these chemicals are found only in areas of current or former intensive use. A second group of compounds such as polychlorinated biphenyls (PCBs), DDT-related compounds (DDTs) or the chlordanes are generally found at concentrations one order of magnitude lower than in temperate regions. The first group of compounds has considerably higher vapour pressures than the second. It appears that the spectrum of organochlorinated compounds shifts to more volatile compounds in the North.

The same patterns appears within a group of compounds: Patton et al.(1991) observed that the PCB pattern in the air of Alert in the Canadian high Arctic is dominated by the low chlorinated congeners with the di- and trichlorobiphenyls amounting to about 55 percent of the total PCBs. Similarly, Bidleman et al.(1989) concluded from a comparison of PCC congener distributions in Arctic air and in a technical toxaphene standard, that "the most volatile constituents were preferentially transported to the Arctic".

Example 2: Residue Levels in Seal Blubber

Table 2 lists levels of major organochlorines detected in the blubber of seals from locations covering a range of latitudes from about 50° to 80° N. The European samples are arranged approximately in a north to south order. The samples comprise different species, number of individuals analyzed, year of sampling, sex, age, and nutritional status and were analyzed by different methods in different laboratories.

Table 2: Concentrations of organochlorines in blubber from seals from different locations (in μg/kg blubber)

Location	Species	No.	HCB	ΣHCH	ΣDDT	ΣPCB	Ref.
Svalbard	Pusa hispida	7	21	142	1642	(837)[a]	(Oehme et al., 1988)
Svalbard	Pusa hispida	11	27	97	1226	2535	(Luckas et al., 1990)
SE-Iceland	Phoca vitulina	7	8	17	1546	5219	(Luckas et al., 1990)
Blakeney/E-England	Phoca vitulina	10	4	27	4743	23000	(Law et al.,1989))
North Sea/Germany	Phoca vitulina	32	7	39	3903	85318	(Luckas et al., 1990)
Baltic Sea/Sweden	Phoca vitulina	9	8	90	22498[b]	84682[b]	(Luckas et al. 1990)
Baffin Island/Can.	Pusa hispida	27	27	213	806	495	(Muir et al., 1988)
Barrow Strait/Can.	Pusa hispida	33	26	288	615	486	(Muir et al., 1988)
Weddell Sea/Antar.	Leptonychotes weddellii	4	4	0.7	105	78	(Luckas et al. 1990)

[a] sum of 5 PCB isomers [b] data from Andersson et al.(1988) confirm the levels of PCB and DDT in seals from the Baltic

Nevertheless, a pattern emerges. Compounds with low volatility show a decline in concentration of up to two orders of magnitude from temperate waters to the Arctic ocean. But for more volatile HCB and HCHs there is a tendency of increasing or at least uniform concentration levels as we approach more remote northerly locations. The HCHs levels in seals from Svalbard and the Canadian High Arctic are higher than those from the waters close to source areas (North Sea, Baltic Sea).

Other animal also show this pattern. When analysing burbot from remote locations along a northwesterly transect from northwestern Ontario to the Mackenzie River Delta in Canada Muir et al.(1990a and 1990b) found that the less volatile compounds DDT, dieldrin, and mirex showed a significant decline in concentration in fish liver with increasing northern latitude, but no decline was observed for HCB, HCHs and toxaphene. Similarly, Andersson and coworkers (1988) concluded from studies on the levels in fish, birds and seals from the northwestern hemisphere: "No pronounced geographical differences were found for PCC when comparing animals from the Arctic region with corresponding species in the Baltic, while both PCB and DDT showed definite spatial differences".

Example 3: Deposition on Plant Surfaces

Calamari et al.(1991) sampled lichens and mango leaves from numerous locations all over the world covering a latitudinal range from Svalbard to Antarctica. Figure 1 shows the concentration of HCB found on plant surfaces as a function of latitude. As can be seen the HCB concentrations in tropical areas are always negligible and increase towards polar regions. Relatively high values in low latitudes are only reported in high altitude samples from Nepal and Kenya.

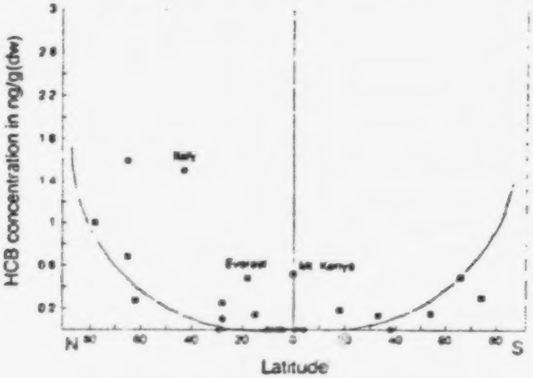

Figure 1

PARTITIONING AND TEMPERATURE

Perhaps these observations provide insights into the mechanisms of transport of these pollutants into polar regions and in the global dispersion and distribution of organic pollutants in general. In the last 20 years it was found that the environmental behaviour and fate of a organic chemical can be described in terms of its partitioning behaviour between separate environmental phases. Multimedia fate models have been developed, which divide the environment in distinct compartments such as air and water (Mackay, 1991). The partitioning behaviour comprises two aspects: The equilibrium distribution between phases expressed as partitioning coefficients and the rate of equilibration between phases expressed as mass transfer coefficients. The partitioning behaviour is characterized by physico-chemical parameters such as vapour pressure, water solubility and the octanol-water partition coefficient K_{ow}.

Multimedia models may be applied to a so-called unit world, i.e. a generic environment with a defined set of parameters, typically containing air, soil, water and sediment phases, which are linked through various transport processes. Advective in- and outflows and degrading reaction can also be incorporated (see Figure 2).

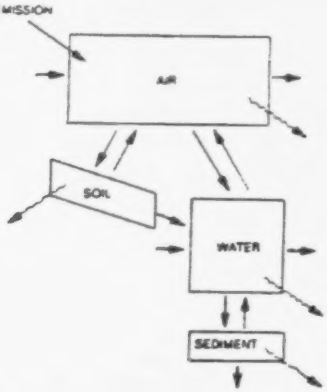

Figure 2

Many partition equilibria are highly dependent on temperature. Bidleman et al. (1987) for example measured the fraction of a chemical which is adsorbed to aerosol particles at different ambient temperatures and found it strongly related to temperature. When determining the Henry's law constant, i.e. the air-water partition coefficient, of γ-HCH Kucklick et al. (1991) found a five-fold decrease in H between 25 and 0°C. Several workers (Hoff et al., 1991, Manchester-Neesvig and Andren, 1989) reported that air concentrations of PCBs and other organochlorines have a pronounced annual variability linked to temperature.

SIMPLE MODEL CALCULATIONS

Multimedia models may be helpful in obtaining a comprehensive picture of the overall effect of changing temperatures. A knowledge of the temperature dependence of physico-chemical properties is required in order to quantify the influence of temperature, but there are few such data available. We have estimated vapour pressures and solubilities of three chemicals at 0 and -25°C by extrapolations in order to perform some basic calculations. At 25°C the vapour pressures of the selected compounds cover a range of 4 orders of magnitude:

Table 3		unit	+25°C	0°C	-25°C
4-Monochlorobiphenyl	vapour pressure	Pa	0.175	6.071e-3	1.071e-4
	solubility	g/m^3	1.2	0.4	0.1
γ-HCH	vapour pressure	Pa	8.589e-3	2.045e-4	2.293e-6
	solubility	g/m^3	6.5	0.85	0.1
DDT	vapour pressure	Pa	5.147e-5	7.156e-7	4.2e-9
	solubility	g/m^3	0.0031	0.002	0.001

We first calculated the equilibrium distribution of these three chemicals in a unit world environment (Level II Fugacity calculation, see Mackay, 1991) at three different temperatures (+25, 0 and -25°C) to see into which compartments a compound preferentially partitions at different temperatures. The parameters of the evaluative environment are described elsewhere (Mackay et al. 1991, the only parameters changed were the land-water distribution of 1:1 and an increased water residence time of 1 year). The degradation of all three compounds was assumed to be slow, i.e. with reaction half lives in the range of years. The compounds partitioned as follows:

Table 4		air	water	soil	sediment	advective residence time
4-Monochlorobiphenyl	+25°C	18.9 %	17.4 %	53.1 %	10.6 %	22 days
	0°C	2.59 %	20.9 %	63.7 %	12.8 %	146 days
	-25°C	0.22 %	21.4 %	65.3 %	13.1 %	851 days
γ-HCH	+25°C	0.88 %	56.7 %	35.3 %	7.1 %	271 days
	0°C	0.18 %	57.1 %	35.5 %	7.2 %	491 days
	-25°C	0.04 %	57.2 %	35.6 %	7.2 %	588 days
DDT	+25°C	0.17 %	1.1 %	82.3 %	16.5 %	5 years
	0°C	0.04 %	1.1 %	82.4 %	16.5 %	9 years
	-25°C	0.03 %	1.1 %	82.4 %	16.5 %	15 years

The influence of temperature is most evident for the Monochlorobiphenyl (MCBP), in which the amount in air drops from 19 percent at 25°C to only 0.2 percent at -25°C. The same phenomenon can be observed for γ-HCH, but to a much smaller extent, because the amount partitioning into air is already relatively small at higher temperatures. The compartmental distribution of DDT is less affected by temperature.

The average residence time of a chemical is strongly dependent on the percentage found in the mobile phases air and water. The shift in distribution resulting from a cooling from +25 to -25°C caused the advective residence time to increase by a factor of 40 for MCBP, and even the small change in the distribution of γ-HCH and DDT translates in a substantial increase in the advective residence time. These simple calculations show that low temperature favours partitioning from the air to the condensed phases water, soil and sediment and thereby prolonging the chemical's residence time in the area.

In a second calculation we explored what happens, if environments with different prevailing temperatures are connected by advective air currents, e.g. by taking the air concentration of one unit world as the input air concentrations for another one. A model was developed where three unit worlds at different temperatures are connected this way. The input of chemical was assumed to occur only into the air compartment of the warmest environment. We now also allowed for deviation from equilibrium partitioning by incorporating rates of intercompartmental exchange processes (Level III Fugacity calculation, see Mackay, 1991). With the exception of temperature all three unit worlds were identical. An iteration was performed to achieve steady-state.

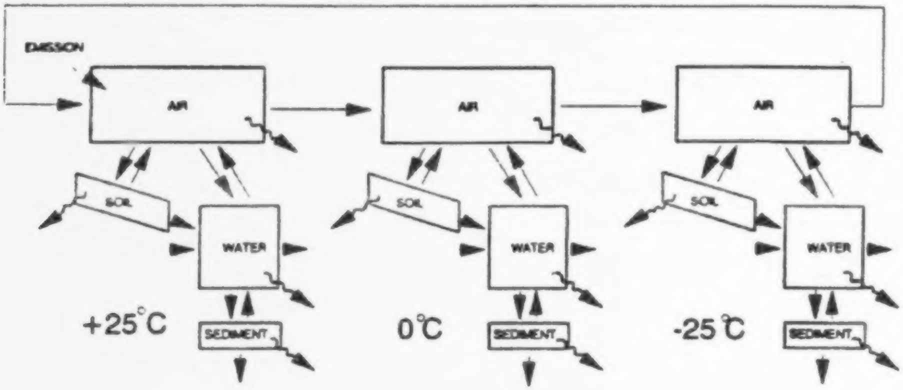

Figure 3

Air concentrations for all three compounds were calculated to decrease towards the colder environments, whereas the concentrations in water, soil and sediment generally are higher in the colder than in the warmer unit worlds (Table 5). The concentrations of DDT also decrease in the condensed phases. For the volatile MCBP the drop in air concentration is relatively small and the increase of the soil concentration is most pronounced.

Table 5	air concentrations in ng/m^3 at			soil concentrations in ng/g at		
	+ 25°C	0°C	- 25°C	+ 25°C	0°C	- 25°C
MCBP	4.13	3.61	2.39	0.013	0.068	0.705
γ-HCH	2.53	1.51	0.36	0.178	0.616	1.034
DDT	1.97	0.52	0.13	5.034	4.729	1.280

Interesting is the overall distribution of the chemicals among the three environments once steady-state is obtained.

Table 6	+25°C-unit world	0°C-unit world	-25°C-unit world
Mono-Cl-Biphenyl	6.2 %	21.1 %	72.7 %
γ-HCH	16.9 %	34.2 %	48.9 %
DDT	46.9 %	41.8 %	11.3 %

As can be seen the relatively volatile MCBP accumulates mostly in the coldest environment - more than 70 % can be found there. γ-HCH is spread more evenly among the different environments, but the colder environments also contain a larger share of the chemical burden. DDT, the least volatile among the three, however, accumulates preferentially in the warmer environments, suggesting that it becomes immobilized by condensation at higher temperatures than the other two substances.

CONCLUSIONS FROM THE MODEL CALCULATIONS

We do not claim that these simple calculations describe accurately the transport of low volatility compounds into the North, but they demonstrate conceptually the effect of temperature on the overall behaviour of these type of chemicals. Obviously, the simulations confirm the suggestions of a "global distillation/cold trap" phenomenon suggested by Goldberg, Ottar and others. High temperatures at the sources of emission favour partitioning into the atmosphere with potential for long range transport, whereas low temperatures in the Arctic favour condensation onto aerosol particles, deposition onto land, into water and ultimately into lipid phases. A systematic transfer of the more persistent compounds from warmer to colder regions occurs (Ottar, 1981), leading eventually to an accumulation of these substances in the temperate and Arctic regions.

The term "global distillation" was used by Goldberg (1975) to describe the transfer of DDT from continents to oceans in the atmosphere and was later extended to include the transport from warmer to colder regions. It has, in our opinion, more subtle features. Compounds behave quite differently according to their volatility resulting in a fractionation as in a distillation. Depending on their vapour pressure, compounds reach an "environmental condensation point" at different ambient temperature levels. The more volatile a compound, the easier and further it travels to remote polar regions. A very involatile substance such as benzo-a-pyrene may hardly leave the region of its emission at all. With increasing volatility the range of transport increases and the further north a compound may condense and accumulate. Very volatile compounds such as the CFCs will never encounter environmental temperatures that would cause them to condense appreciably. Our calculations suggest that condensation begins approximately when the vapour pressure drops below 10^{-2} to 10^{-3} Pa.

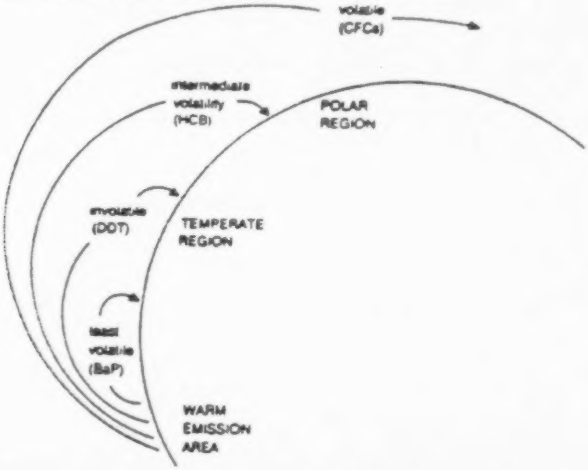

Figure 4

An experimental confirmation of a selective condensation of the less volatile constituents of a mixture is reported by Larsson and Okla (1989). In Sweden they analyzed simultaneously PCB patterns in the lower atmosphere and in atmospheric fallout and found the lower chlorinated congeners enriched in the atmosphere, while the higher chlorinated ones dominated in the fallout. The introductory examples are also in accordance with the implications derived from our calculations. The less volatile organochlorines such as DDT, chlordane and dieldrin show a steep gradient in concentrations as we move towards northern latitudes, while the more volatile compounds show a uniform or even reversed concentration profiles as they do in our model outputs.

PROSPECTS

The models presented here are very simple. The unit world used is essentially a typical temperate environments. Snow and ice are not included. The changes in environmental parameters such as precipitation and sedimentation rates or the organic carbon content of soils and sediments, that occur when moving from a temperate to a polar environment are not taken in consideration. Furthermore, degradation rates in the various media will change considerably in different environments.

We are currently developing refined models to account for these shortcomings. These will include the calculation of concentrations in biological samples. Major knowledge gaps that have to be overcome in this attempt are: accurate information on the temperature dependence of the physical-chemical properties of the organochlorine pollutants, an understanding of the role of snow and ice, especially the exchange processes with the atmosphere, and a general knowledge of Arctic ecosystems such as water and sediment balances, the composition of the various media (e.g. organic carbon content), food web structures, and many other things.

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SELECTIVE ACCUMULATION OF POLYCHLOROCAMPHENES IN AQUATIC BIOTA FROM THE CANADIAN ARCTIC.

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INTRODUCTION

Toxaphene was at one time the most heavily used insecticide in the United States. Applied largely in the "cotton belt" of the southern U.S. and also for diverse purposes in other locations, toxaphene use peaked between 1972-75 at 25,000 - 37,000 tonnes/y (Bidleman et al., 1988; Voldner and Schroeder, 1989). Toxaphene underwent a decline in the late 1970s and early 1980s, and was banned in 1982 with the stipulation that existing stocks could be applied through 1986. Toxaphene-like products have also been used in Mexico, eastern Europe and the Soviet Union (Bidleman et al., 1988; Voldner and Schroeder, 1989).

Toxaphene is produced by chlorinating camphene to about 67-69% Cl content, which yields a complex mixture of mainly bornanes and bornenes substituted with 6 to 10 chlorines (Swackhamer et al., 1987)(Figure 1). Some foreign products are made by chlorination of terpenes. Generically, toxaphene and similar products are known as "polychlorocamphenes" (PCCs) or "polychloroterpenes".

It is now recognized that PCCs have been globally dispersed to the same extent as DDT and PCBs. In North America PCCs are major organochlorine compounds (OCs) in Great Lakes fish (Swackhamer and Hites, 1988; Evans et al., 1991), fish and marine mammals from the Canadian Arctic (Muir et al., 1990, 1991), and peat cores from several locations near the eastern U.S. - Canadian border (Rapaport and Eisenreich, 1988). PCCs are also abundant in fish and seal from Scandinavia (Andersson et al., 1988; Passivirta and Rantio, 1991) and the North Sea (van der Valk and Wester, 1991).

In the course of examining PCC residues in arctic biota, we noticed greatly altered gas chromatographic patterns in samples compared to a toxaphene standard. In some cases these differences were so pronounced that identification by electron capture gas chromatography (GC-ECD) alone was problematic, and it was only through GC with electron impact or negative ion mass spectrometry (GC-EIMS, GC-NIMS) that the presence of PCCs could be established with confidence (Bidleman et al., 1989; Muir et al., 1990, 1991). Such differences in GC patterns might be caused by selective accumulation of certain PCCs, either by differential uptake or metabolism. Here we report examples of selective PCC accumulation in the liver of a fresh water fish (burbot, Lota lota) and narwhal (Monodon monoceros) blubber.

METHODS

Samples of burbot liver and narwhal blubber were from specimens collected in the Canadian Arctic by Muir et al. (1990, 1991). Details of extraction and analytical methods for PCCs and other OCs are given in other publications (Patton et al., 1989; Muir et al., 1990, 1991). Briefly, tissue extracts were freed from lipids by gel permeation chromatography and sulfuric acid cleanup, and the samples were then fractionated on florisil. GC-NIMS analysis was carried out in the full-scan mode on a 30-m DB-5 fused silica capillary column using a Finnigan 4521C instrument.

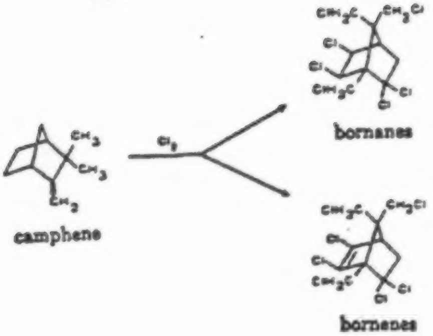

Figure 1. Production of representative chlorinated bornanes and bornenes from camphene. From Swackhamer et al. (1987).

RESULTS AND DISCUSSION

Levels of PCCs and other OCs in burbot liver and narwhal blubber from the Canadian Arctic, summarized from Muir et al. (1990, 1991), are given in Table 1. PCCs outrank other OC pesticides and even PCBs in abundance.

PCCs fragment under NIMS conditions to yield mainly $(M-Cl)^-$ species (Swackhamer et al., 1987). Figure 2 shows chromatograms of a toxaphene standard, burbot liver, and narwhal blubber, obtained by plotting the sum of the following $(M-Cl)^-$ ions: 7-Cl = 343,345; 8-Cl = 379,381; 9-Cl = 413,415. Differences between PCC patterns in the biological samples and standard are striking. In this report we focus on the two prominent peaks, 1 and 2, which have been also designated as T1 and T12 by Muir et al. (1991). The relationship of these peaks to other OCs (PCBs, chlordanes, DDTs) are shown in GC-ECD and GC-NIMS chromatograms in Muir et al., 1990, 1991). The burbot and narwhal extracts were fractionated on florisil, and PCCs 1 and 2 divided between the two fractions as shown in Figure 2. PCC 2 was also the major PCC in fraction 2 of benthic amphipod samples from the Canadian Ice Island (Bidleman et al., 1989).

Table 1. Organochlorine Compounds[1] in Burbot Liver and Narwhal Blubber.

	PCCs	PCBs	HCB	DDTs	CHLORs	Dieldrin
Burbot liver[2] ng/g lipid	807-2338	301-1941	24-66	51-1490	86-378	7-71
Narwhal blubber[3] ng/g wet wt.						
males	2990-13200	2250-7290	209- 911	2600-8560	939-2510	268-667
females	1910-8390	894-5710	137-1140	595-5910	546-2820	155-832

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1. PCCs = polychlorocamphenes, PCBs = polychlorobiphenyls, HCB = hexachlorobenzene, DDTs = sum of DDT-group compounds (isomers of DDT, DDE, DDD), CHLORs = chlordanes + nonachlors.

2. Range of geometric means at different locations. 3. Range.

Toxaphene contains over 200 components, and separation by capillary GC on a 30-m column is incomplete. Peaks 1 and 2 appear to contain a mixture of PCCs in both the standard and samples. Figure 3 shows spectra obtained from narwhal: PCC 1 florisil fraction 1, and PCC 2 florisil fraction 2. Although these PCCs split on the florisil column, there are no great differences in the spectra of 1 and 2 in the two fractions. Also, spectra of PCCs 1 and 2 from narwhal and burbot are similar.

Considering PCC 1 in the sample and standard, the spectra are consistent with the peak being a mixture of 7-Cl and 8-Cl bornanes. Base $(M\text{-}Cl)^-$ ions for these are 341 and 375, respectively. A co-eluting hexachlorobiphenyl can also be seen in the narwhal sample from the cluster having $M^- =$ 358. The 8-Cl PCC(s) are clearly enriched in the narwhal. The small 7-Cl remnant in the sample has a slightly different spectrum from the similar cluster in the standard. In particular, the ion at 339 and enhancement of 341 relative to 343 may be due to $(M\text{-}Cl)^-$ and higher ions from a heptachlorobornene. Another possibility is production of $(M\text{-}Cl{+}O)^-$-type ions from hexachlorobiphenyl as a result of traces of oxygen in the source (van der Valk and Wester, 1991). However we ran GC-NIMS scans of Aroclor 1254, and these failed to show any traces of such ions.

PCC 2 is a mixture of 7-Cl, 8-Cl, and 9-Cl components, as seen from Figure 3 and also from the greatly expanded scale in Figure 4. Out of this mixture the narwhal has selectively accumulated a narrower range of compounds, dominated by 9-Cl PCCs (Figure 3 and 4). This preference was also

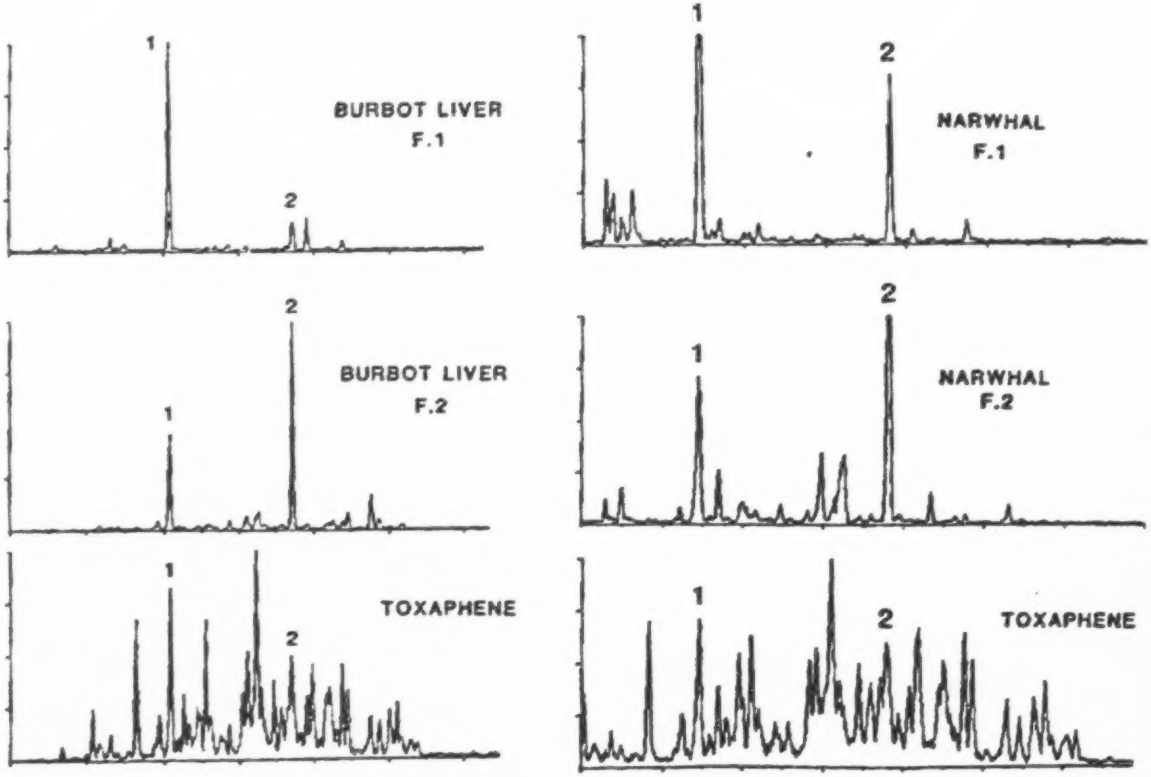

Figure 2. GC-NIMS chromatograms (sum of ions 373, 375, 379, 381, 413, 415) of burbot liver, narwhal blubber, and a toxaphene standard.

shown by amphipods from the Ice Island (Bidleman et al., 1989). As for PCC 1, ions at 339 and 373 may signal $(M-Cl)^-$ of bornene components.

PCCs are delivered to the arctic through the atmosphere, and polar air and snow samples show the presence of PCCs (Bidleman et al., 1989; Hinckley et al., 1991; Patton et al., 1989, 1991). PCC profiles in air, water, and zooplankton are similar to each other and differ from a toxaphene standard mainly in the predominence of lighter (more volatile) constituents. Thus physical "weathering" alone probably cannot account for the extreme chromatographic differences in Figure 2. Selective accumulation through differential uptake and metabolism of certain PCCs is more likely, as suggested by van der Valk and Wester (1991).

Pure PCB congeners have been on the market for years, and these have greatly aided the development of PCB analytical chemistry and toxicology. Unfortunately we are in the same position with toxaphene as for PCBs in the early 1970s — only the technical mixture is available. The abundance of PCCs in arctic food chains and the fact that these residues are obviously not well represented by technical toxaphene requires that pure PCC congeners be isolated (or synthesized) and evaluated for analytical, physical, and toxicological properties.

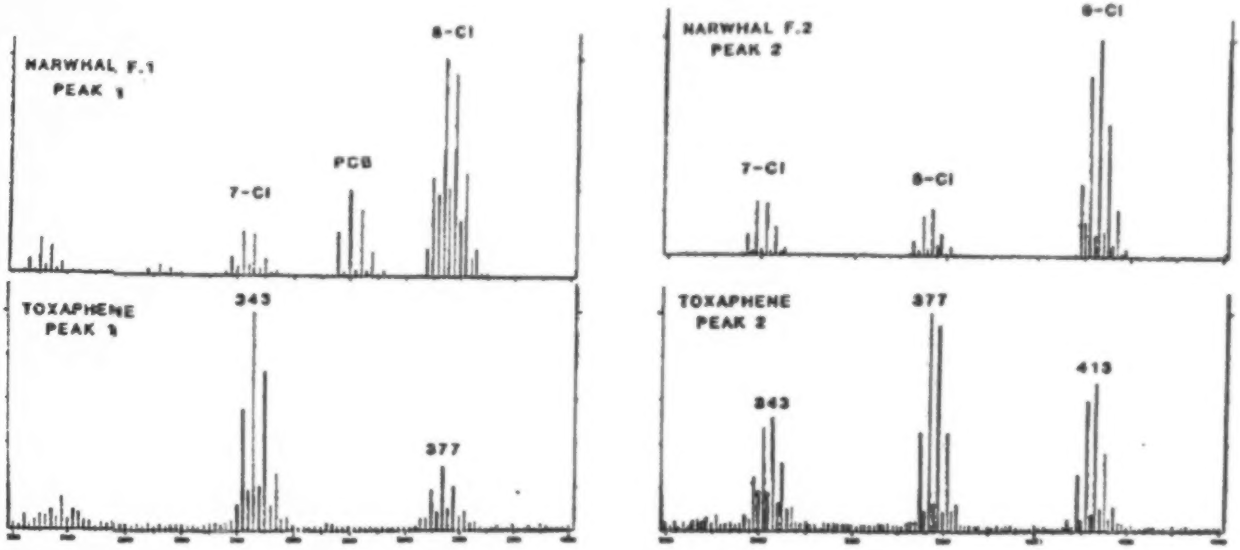

Figure 3. Narwhal extract. left: spectra of PCC peak 1 in florisil fraction 1 compared to the same peak in a toxaphene standard. right: PCC peak 2, florisil fraction 2 compared to the average spectrum of peak 2 in the standard.

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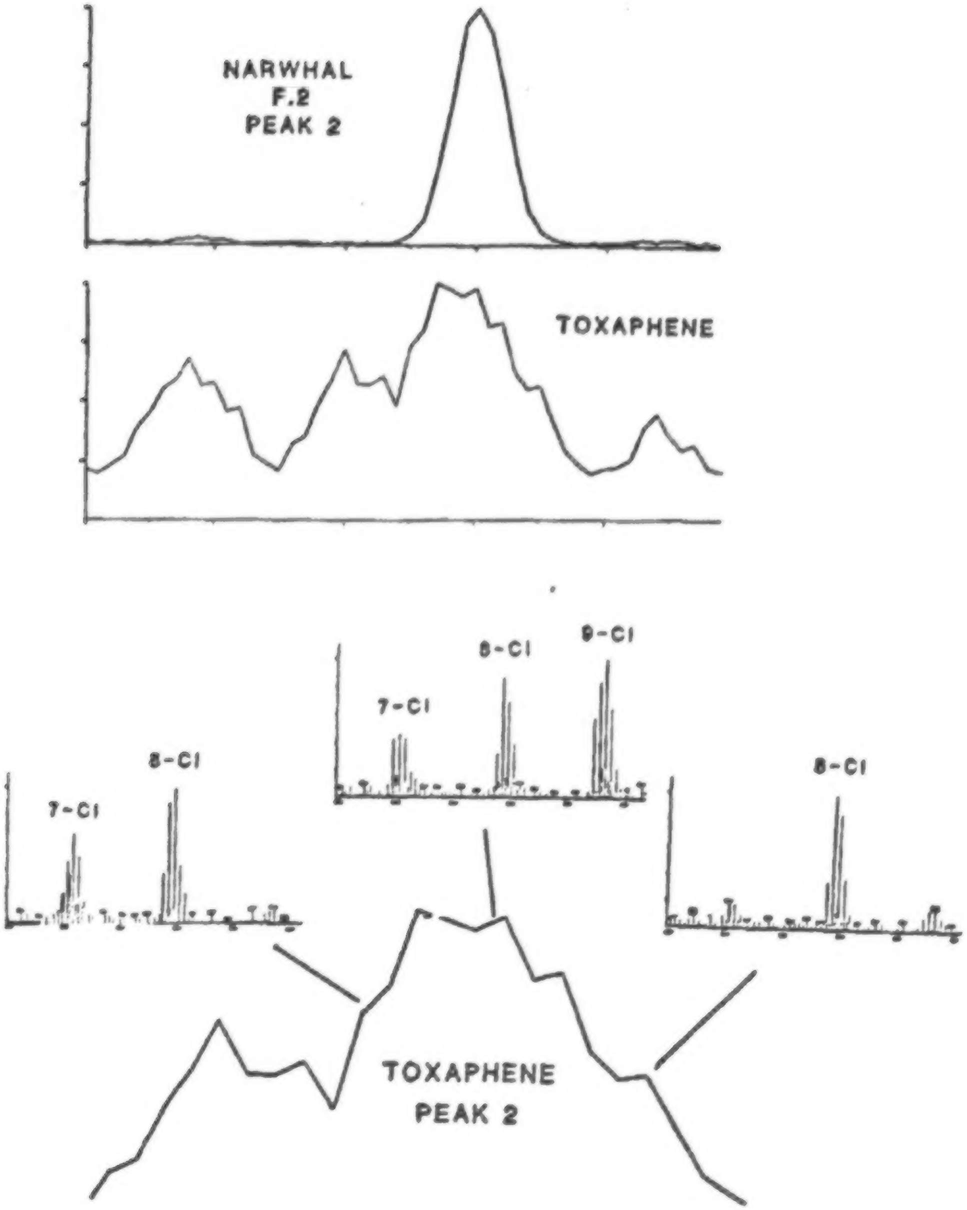

Figure 4. Expanded scale of the peak 2 region of narwhal and the toxaphene standard. Spectra below show compositional changes over different parts of peak 2 in the standard.

PCB METHYL SULPHONES IN MAMMALS FROM CANADIAN AND SWEDISH ENVIRONMENTS.

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Introduction

Polychlorinated biphenyls (PCB) are ubiquitous environmental contaminats with marked biomagnification properties and are present in environmental samples at similar concentrations as ΣDDT. The monitoring of PCB and DDT levels in various environmental samples has been extensive [1]. In contrast, information about the presence and concentrations of persistent metabolites of PCB and DDT, PCB methyl sulphones ($MeSO_2$-PCB) and DDE methyl sulphones ($MeSO_2$-DDE), respectively, in the environment is scarce. $MeSO_2$-PCB and $MeSO_2$-DDE were first detected in blubber of grey seal from the Baltic [2]. Subsequently, the presence of $MeSO_2$-PCB in different organisms and humans from the japanese environment [3,4] and in otter, harbour seal and ringed seal from the swedish environment [5] was reported. Recently the synthesis of a large number of individual methylsulphonyl-substituted chlorinated biphenyls ($MeSO_2$-CBs) [6] have made identifications and quantifications of these PCB metabolites possible.

Aryl methyl sulphones are formed via nucleophilic attack by glutathione on an arene oxide intermediate formed in the first step of metabolism [7,8]. The glutathione conjugate formed is degraded to the corresponding cystein conjugate and further to the aryl thiol after cleavage of the cystein C-S bond by ß-lyase [8]. The aryl thiol is methylated and the aryl methyl sulphide formed is subsequently oxidized to form the corresponding aryl methyl sulphone. The retention of $MeSO_2$-CBs in environmental samples and more, the specific tissue localizations of certain $MeSO_2$-CBs and $MeSO_2$-DDE to specific tissues have initiated futher studies of these compounds [9]. This can be exemplified with 4-$MeSO_2$-2,4,2',5'-tetrachlorobiphenyl that is strongly retained in the broncial mucosa in mice [8]. A similar retention has been observed for some other *para*-substituted $MeSO_2$-CBs [10]. The retention was shown to be due to the binding of these $MeSO_2$-CBs to a uteroglobin-like protein present in mouse, rat and human lung [11]. However, the toxicological significance of the $MeSO_2$-CBs has not yet been determined. In contrast, $MeSO_2$-DDE has been shown to be highly toxic to mice and cause severe damage, necrosis, in *zona fasiculata* in the adrenal cortex.

In the present study analysis for $MeSO_2$-PCB and $MeSO_2$-DDE in samples from polar bear (*Thalarctos maritimus*), beluga whale (*Delphinapterus leucas*) and false killer wahle (*Pseudorca crassidens*) from the Canadian environment and grey seal (*Halichoerus grypus*), otter (*Lutra lutra*) and wild mink (*Mustela vison*) from the Swedish environment are reported.

Material and Methods

Chemicals: The CB methyl sulphone congeners ($MeSO_2$-CBs) used as reference compounds and as the internal standard (IS), 3-methylsulphonyl-4-methyl-2',3',4',5,5'-pentachlorobiphenyl, were prepared by Haraguchi *et al.* [6]. All solvents were of analytical grade or treated, prior to use, as described elsewhere [13]. Aluminum oxide (neutral, activity grade I) from Merck (Darmstadt, Germany) was used for column chromatography [5]. All glass were heated at 300°C over night before they were used [13].

Instruments: The $MeSO_2$-CBs were analyzed on a Varian 3400 gas chromatograph (GC) equipped with an autosampler (Varian model 8100) and an electron capture detector (ECD, ^{63}Ni). The chromatographic data were recorded by a data system (ELDS, Chromatograpy Data Systems AB, Kungshög, Sweden). The GC was fitted with a fused silica capillary column HP-5 (50 m x 0.2 mm i.d. and 0.33 µm film thickness) from Hewlett Packard. The injections were made in the splitless mode (injector temperature: 250°C) and the GC temperature program was: 70°C (2 min), 25°C/min to 230°C, 4°C/min to 280°C (40 min). Hydrogen was used as carrier gas and nitrogen as make up gas. The detector temperature was 360°C. Mass spectrometry was performed with a Finnigan ITS 40 benchtop mass spectrometer with GC separations performed on a Varian 3400 instrument equipped with a fused silica capillary column BP 5 (30 m x 0.25 mm i.d.) from J&W Scientific Inc, with helium as carrier gas. The temperature program was: 70°C (2 min), 20°C/min to 220°C (10 min) and then 3°C/min to 280°C (16 min). The injections were made in the splitless mode, using an autosampler (CTC A200S, FinniganMAT) with an injector temperature of 260°C. The mass spectrometer was operated in the electron impact mode (EI) and the manifold temperature was 220°C.

Samples: Lipid extracts previously prepared for analysis of other organochlorine compounds were used [14]. Extracts from bubber and liver of polar bear and beluga whale, blubber from grey seal and false killer whale and adipose tissue from mink and otter were analyzed.

Analysis: The lipid extracts (approx. 2 g) were dissolved in hexane and partitioned with anhydrous dimethyl sulphoxide (DMSO) as described elsewhere [13]. To each extract, 600 ng of the IS was added before any further clean-up was carried out. The $MeSO_2$-CB fraction, in hexane, was further purified by partitioning between hexane and sulphuric acid and finally by liquid chromatography on aluminum oxide [5]. All samples obtained were analyzed by GC(ECD) and subsequently by GC/MS.

Results and Discussion

Gas chromatograms of $MeSO_2$-CBs and $MeSO_2$-DDE from the purified lipid extraxts of polar bear and grey seal are shown in figure 1 and 2, respectively. The numbers in the chromatograms refer to the tentatively identified $MeSO_2$-CBs listed in table 1. In table 1, also the compounds identified in the different species analyzed and in different tissues as well as the total concentrations of $MeSO_2$-CBs in the samples analyzed are shown. The concentrations of $MeSO_2$-CBs were too low for GC/MS analysis of the beluga whale and false killer whale samples. No $MeSO_2$-CBs were identified in the false killer whale due to low concentrations of potential $MeSO_2$-CBs and different retention times of compounds present in the sample compared to other samples analyzed. The present study may be regarded as a preliminary investigation on quantification of these $MeSO_2$-substituted xenobiotics since only one or two individual samples were analyzed of each species or tissue. $MeSO_2$-PCB are present in the low ppm range with 10 - 30 times higher concentrations of PCB in the samples also analyzed for PCB. This is in agreement with other data from analysis of mammals [5].

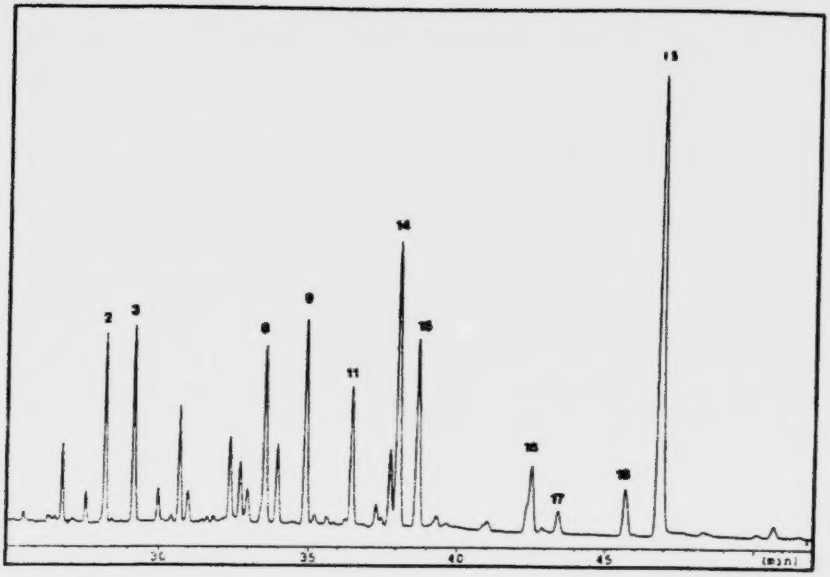

Figure 1. Gas chromatogram of $MeSO_2$-CBs and $MeSO_2$-DDE in polar bear blubber. The numbers correspond to the structures given in table 1. IS indicate internal standard.

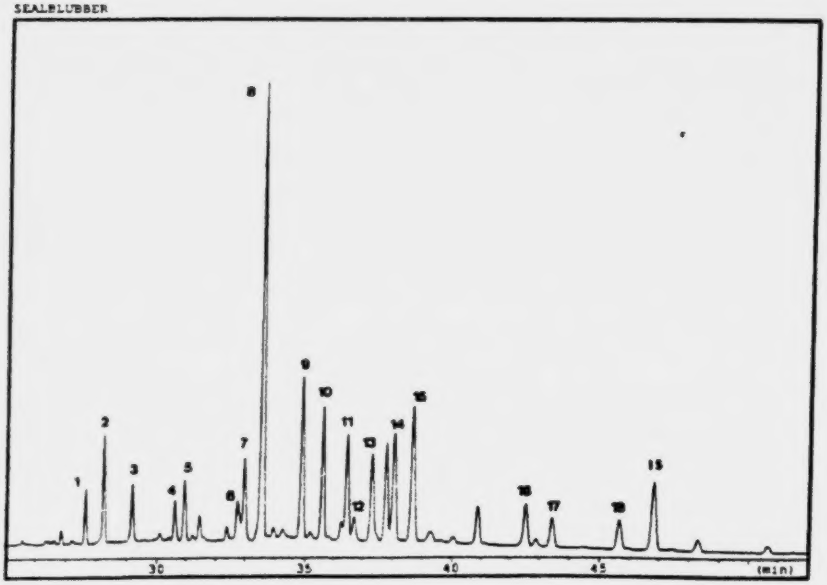

Figure 2. Gas chromatogram of $MeSO_2$-CBs and $MeSO_2$-DDE in grey seal blubber. The numbers correspond to the structures given in table 1. IS indicate internal standard.

Table 1. Tentative structures (+) of $MeSO_2$-CBs, based on similar GC(ECD) and GC/MS properties as the corresponding authentic reference compounds, and concentration of total $MeSO_2$-CBs and $MeSO_2$-DDE isomers in the samples analyzed are shown.

peak no.	Structures	PB[1] blubber	PB liver	BW[2] blubber	BW liver	FKW[3] blubber	WM[4] adip. tiss.	O[5] adip. tiss.	GS[6] blubber
1	3-$MeSO_2$-2,5,2',5'-tetraCB							+	+
2	3-$MeSO_2$-2,5,2',4'-tetraCB	+	+	+	+				+
3	4-$MeSO_2$-2,5,2',4'-tetraCB	+	+	+	+			+	+
4	3-$MeSO_2$-2,5,6,2',5'-pentaCB								+
5	2-$MeSO_2$-DDE (µg/g l.w.)	nd[7]	0.9	nd	nd	0.1		+	0.2
6	3-$MeSO_2$-2,5,3',4'-tetraCB + $MeSO_2$-pentaCB	+		+	+				+
7	Unknown		+				+		+
8	3-$MeSO_2$-2,5,2',4',5'-pentaCB	+	+	+	+		+	+	+
9	4-$MeSO_2$-2,5,2',4',5'-pentaCB	+	+	+	+		+	+	+
10	3-$MeSO_2$-DDE (µg/g l.w)	nd	0.4	nd	nd	0.1	+	0.01	0.5
11	3-$MeSO_2$-2,5,2',3',4'-pentaCB	+	+	+	+		+	+	+
12	3-$MeSO_2$-2,5,2',3',5',6'-hexaCB								+
13	4-$MeSO_2$-2,5,2',3',5',6'-hexaCB		+				+	+	+
14	4-$MeSO_2$-2,5,2',3',4'-pentaCB	+	+	+	+		+	+	+
15	4-$MeSO_2$-2,3,6,2',4',5'-hexaCB	+	+				+	+	+
16	4-$MeSO_2$-2,3,6,2',3',4'-hexaCB	+	+				+	+	+
17	3-$MeSO_2$-2,5,2',3',4',5'-hexaCB	+	+	+	+				+
18	4-$MeSO_2$-2,5,2',3',4',5'-hexaCB	+	+	+	+			+	+
	Total $MeSO_2$-CB concentration (µg/g l.w.)	1.0	4.6	0.7	1.0	-	0.2	0.4	8.1

1) polar bear, 2) beluga whale, 3) false killer whale, 4) wild mink, 5) otter, 6) grey seal, 7) nd = not detected

Remarkably, the liver samples contain higher concentrations of $MeSO_2$-CBs, on a lipid weight basis, than the adipose tissue samples indicating a different mechanism in the retention of $MeSO_2$-CBs in those tissues. The difference is most obvious in livers from grey seal [5], mink and otter where a few $MeSO_2$-CBs are present in high concentrations. These observations are in accordance with data recorded from an experiment where minks were dosed with PCB (Clophen A50) [13]. This indicate that certain structures of $MeSO_2$-CBs can bind non-covalently to specific protein sites in the liver. Protein binding of this type has been described not only for the above mentioned uteroglobin type protein but also for the binding of a bis-$MeSO_2$-tetraCB to a fatty acid bindig protein [14].

Two isomers, 2-$MeSO_2$-DDE and 3-$MeSO_2$-DDE, are formed from DDE. Both isomers are present in the polar bear liver, false killer whale and grey seal blubber samples and the concentrations of the two isomers are shown in table 1. The ratio of 2-$MeSO_2$-DDE/3-$MeSO_2$-DDE varies with species and tissue. 2-$MeSO_2$-DDE is the major isomer in polar bear liver while it is the minor isomer in grey seal blubber [5]. $MeSO_2$-DDE could not be detected in beluga whale and were present only in trace amounts in adipose tissue from mink and otter. The peak no 7, a compound present in grey seal blubber (figure 2), but a major compound in polar bear liver, referred to as unknown, correspond to a $MeSO_2$-substituted substance with a M^+ of 362, with an isotopic ratio corresponding to the presence of 3 chlorine atoms in the molecule, and a fragmentation pattern that indicate a dechlorinated $MeSO_2$-DDD structure.

The structures of the parent CBs to at least some of the identified $MeSO_2$-CBs can be deduced. Thus, 2,4,2',5'-tetraCB, 2,4,5,2',5'-pentaCB, 2,3,4,2',5'-pentaCB and 2,3,6,2',4',5'-hexaCB can be identified as some of the CBs that are metabolized to the $MeSO_2$-CBs shown in table 1. CBs with a 2,5-dichloro- or a 2,3,6-trichloro-substituted phenyl thus having an unsubstituted *meta-/para*-position are most easily transformed to $MeSO_2$-CBs. In the environment, the decrease in concentrations of many of the more easily transformed CBs correspond to an increase in the levels of $MeSO_2$-CBs. As stated above, the toxicological significance of $MeSO_2$-CBs is unknown but since the levels in the environment are as high as shown in the present paper further work is nessessary to form a better understanding with regard to the toxicity of these environmental contaminants.

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POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS FROM ARCTIC LAKES AND MIXED-FUNCTION OXIDASE ENZYMES IN FISH FROM THE SAME LAKES.

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Over the period from 1988 to 1991 sediments and fish have been collected from a series of lakes along a north/south transect extending approximately from the U.S. border (latitude 49 N) to northern Ellesmere Island (latitude 82 N). The purpose of work has been to determine rates of deposition of air-borne contaminants (organochlorines, metals, PAHs) from analyses of sediment core slices, and to determine whether any changes in the biochemistry of fish can be detected consistent with the histories of contaminant inputs as determined from the cores. This presentation will focus on a description of 'priority' polycyclyc hydrocarbons in the sediments from the four locations, and on the microsomal enzyme activities in fish from the same lakes.

Two new box coring devices were built small enough to permit relatively convenient surface transport and operation from ice, but large enough to provide enough sediment for all the analyses. This device has worked successfully in water up to 260 m deep to furnish 10-40 g of dry sediment for analyses. After removal from the water, cores were sliced ~~immediately~~ in ~~the~~ field; sediment layers were placed into separate bottles and were shipped to the Freshwater Institute for analyses. Core slices were analyzed for radionuclides (210-Pb, 137-Cs) to estimate the period of deposition of each slice, for bulk sediment characteristics, and for contaminants. Fish were usually obtained by angling, and were dissected freshly after removal from the water. Livers were fast-frozen on dry ice and shipped in that state to the Freshwater Institute where they were maintained at -80 C until analyzed. No single species of fish was available from all the lakes; we have concentrated on the genus Salvelinus because lake trout (Salvelinus namaycush) was available from southern locations and arctic char (Salvelinus alpinus) from northern ones.

Core slice PAHs (excluding perylene and retene) showed sub-surface maxima at the Experimental Lakes Area (49 N) and at Saqvaqjuac (64 N), implying that maximum inputs occurred during the middle years of the current century. The pre-industrial base at 49 N was substantially above that at 64 N, as was the size of the sub-surface peak. Current inputs were below peak inputs, but still well above the pre-industrial "background". On Cornwallis Island (75 N), the top two slices contained about twice the concentrations of lower slices, suggesting a similar pattern to those in more southern locations, without the recent decline. The pre-industrial levels were lower still than those at either ELA or Saqvaqjuac. At Lake Hazen on Ellesmere Island (82 N) the pattern of PAHs showed a continuous increase since the early 1800s. Qualitatively the Lake Hazen PAHs were rich in perylene and retene, even at the sediment surface, and perylene failed to increase with depth. Examination of the core material microscopically showed granules of unburned coal and amber; collections of these materials from the basin showed PAH profiles rich in perylene and retene. The sources of the high PAH loadings to Lake Hazen were probably a combination of fallout and erosion of coal deposits within the basin.

The mixed function oxidase enzyme activities of the fish from all these areas were very similar. There was no indication that current loadings of PAHs or accumulated loadings over time were related to the enzyme activities in arctic charr or lake trout. Probably the exposure levels are below those required to elicit responses large enough to be distinguished statistically. The highest current loadings were from Lake Hazen on Ellesmere Island, and the failure of even these fish to exhibit any increased enzyme activity suggests that the PAHs associated with coal are not readily available biologically.

BREAST MILK CONTAMINATION BY PCDDs, PCDFs, PCBs, COPLANAR PCBs AND CHLORINATED PESTICIDES IN ARCTIC QUÉBEC.

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In 1988, we found very high levels of PCBs and DDE in the milk of 24 Inuit women of Hudson Bay (Northern Québec). Levels of PCBs in the Inuit breast milk were 5 times those of women from the Southern part of the Province (Dewailly, 1989). In view of these data, we decided with the Kativik Regional Board of Health to conduct a follow-up study of all babies (breast fed and bottlefed) during one year.

The main objectives of this survey are:

1. To measure the biological exposure (breast milk) of this Inuit population (Hudson Bay, Hudson Straight and Ungava Bay) for organochlorines (PCBs, coplanar PCBs, chlorinated pesticides and dioxins/furans).
2. To identify the main factors (personal and dietary) associated with the breast milk levels.
3. To assess health risk for infants by comparing prospectively (at 2, 6 and 12 months) the health status (clinical and biological evaluation) of breastfed and bottlefed babies during one year.

This paper present the data of the 105 breast milk analyses. Results of PCBs, coplanar PCBs, PCDDs/PCDFs and chlorinated pesticides are compared with levels from the Southern part of the Province of Québec.

POPULATION AND METHODS

6500 persons inhabit the 14 settlements scattered along the shoreline of Hudson Bay and Ungava Bay between 55° and 63° North of latitude. Each mother who have delivered between mid July 1989 and mid July 1990 were ask to participate to the study. Among the 224 live births, 113 start to breasfed their babies. Among them only 9 were not join and 3 refused to participate in the study. Each mother provided a 60 ml vials of milk within the first three days following delivery. Milk samples were sent frozen to the Québec Toxicology Center for PCB and chlorinated pesticides analyses (n= 105). 40 sub samples were also sent to the Midwest Research Institute (Kansas City - USA) for coplanar PCB, PCDD and PCDF determination.

Analyses of 10 PCB congeners (IUPAC No 28, 52, 101, 118, 138, 153, 170, 180, 183 and 187) and 8 chlorinated pesticides (HCB, heptachlor epoxide, α and δ chlordane, dieldrin, DDE, endrin, Mirex) were analyzed by HRGC using an electron capture detector. Non-ortho coplanar PCB (IUPAC No 77, 126 and 169) and 2,3,7,8 chloro substituted congeners of PCDDs and PCDFs were analyzed by HRMS/HRGC.

RESULTS

The mean level of PCB (Aroclor 1260) is 2.9 mg/kg (CI 95 %: 2.46 - 3.31) in the milk fat of Inuit women compared with 0.52 mg/kg (CI 95 %: 0.50-0.54) for the Caucasian group (n= 572). PCB congeners are between 3.4 times (No 118) and 10.6 times (No 153) higher in the Inuit group than in the Caucasian group (Figure 1). For non-ortho the coplanar PCBs, levels of the tetra 3,3',4,4'-CB (No 77), the penta 3,3',4,4',5-CB (No 126) and the hexa 3,3',4,4',5,5'-CB (No 169) are respectively 24.8, 209.3 and 220.9 ng/kg (fat basis) for the Inuit group (n= 40) and 8.1, 80.5 and 32.7 ng/kg for the control group (n= 16 pools; 96 milks) (Figure 2).

For PCDDs and PCDFs, differences between the two groups are less impressive. Except for OCDD (292 vs 132 ng/kg) and TCDD which are 2 to 3 times more elevated among Inuit, other congeners are detected at similar levels in both groups. TCDF is 3 times less elevated in the Inuit group (figures 3 and 4). For chlorinated pesticides, differences between milk levels found among Inuit women are 3 to 5 times those of Caucasian women (Table 2).

DISCUSSION AND CONCLUSION

Breast milk levels of PCBs and coplanar PCBs are 6 times more elevated among Inuit women of Northern Québec than among women living in Souhern part of the Province of Québec. Milk levels of PCDDs and PCDFs are not very high in this region but it is possible that the unusual South to North increasing trend observed for these compounds among polar bears could be also observed for humans. Evaluation of PCDDs and PCDFs levels in human should be determined in the high Arctic. Considering a breasfed baby receiving 4.2 gr of milk fat per kg of body weight (120 ml of milk/kg-bw), it is then possible to compare the daily intakes of different contaminants with Acceptable Daily Intakes (ADI) (Table 1). The ratio of daily intake/ADI range between 0.2 and 8 for chlorinated pesticides; 10 for PCBs; 80 for PCDDs/PCDFs and 145 for coplanar PCBs. Considering this specific high exposure of infants the results of the follow-up study will give useful data for managing this public health risk.

TABLE 1: Daily intakes vs Acceptable Daily Intakes of organochlorines for infants in Northern Québec (Organochlorines).

ORGANOCHLORINES	DI (µg/kg-bw)	ADI (µg/kg/bw)	DI/ADI
Heptachlor epox.	0.089	0.5 (EPA, 87)	0.18
HCB	0.45	0.8 (EPA, 89)	0.31
Chlordane	0.22	0.06 (EPA, 90)	3.7
Dieldrine	0.14	0.05 (EPA, 87)	2.8
DDE	4.0	0.5 (EPA, 87)	8
Endrin	0.03	0.3 (EPA, 88)	0.1
PCB 1260	10.50	1 (EPA, 84)	0.5
PCDD/PCDF*	80.6 pg/kg/j	1 pg (EPA,88)	80.6
PCB Coplanar**	145 pg/kg/j	1 pg (EPA,88)	145

* Toxic Equivalent Quantity (NATO 1988), ** Toxic Equivalent Quantity (SAFE 1990)

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NATO: North Atlantic Treaty Organization Committee on the Challenges of Modern Society 1988. Pilot study on international information exchange on dioxins and related compounds. Scientific basis for the development of the International Toxicity Equivalency Factor (I-TEF). Method of risk assessment for complex mixtures of dioxins and related compounds. #178.

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Fig.1: BREAST MILK LEVELS OF PCB CONGENERS

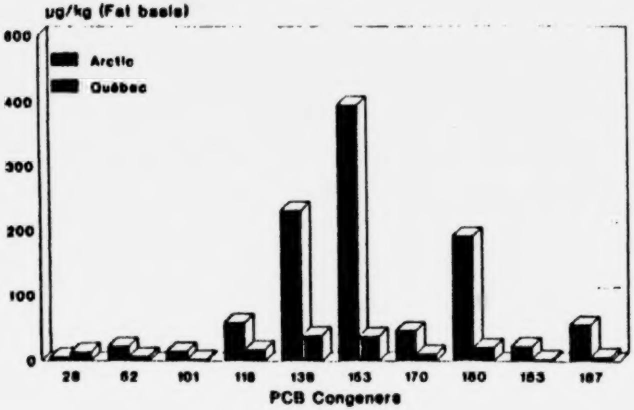

Fig.2: COPLANAR PCBs IN HUMAN MILK
(arith. means, ng/kg fat basis)

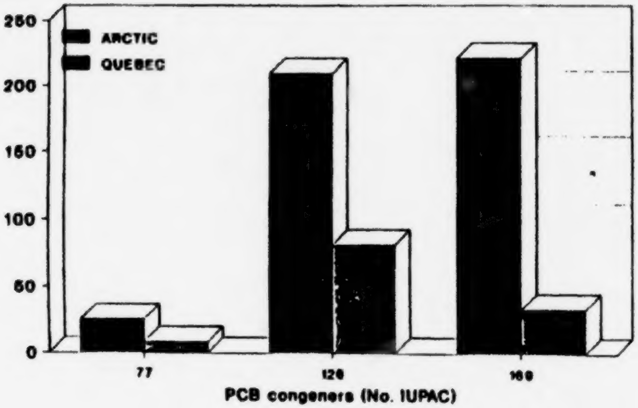

Fig.3: PCDDs IN HUMAN MILK
(arith. means, ng/kg fat basis)

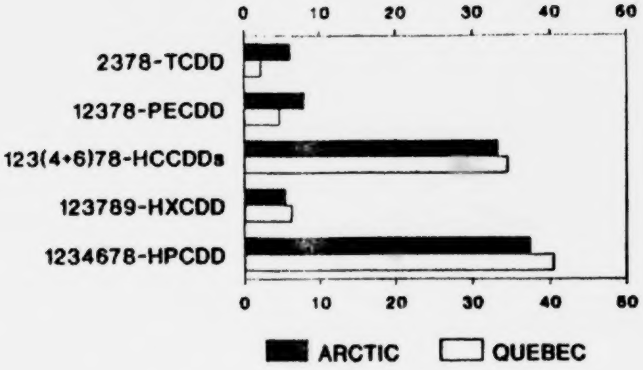

Fig.4: PCDFs IN HUMAN MILK
(arith. means, ng/kg fat basis)

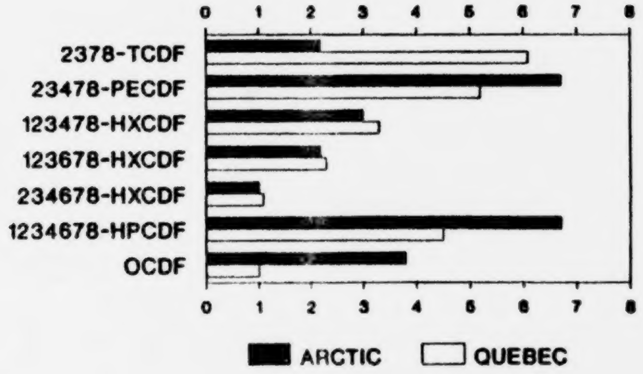

TABLE 2

CHLORINATED PESTICIDES IN THE BREAST MILK OF INUIT AND CAUCASIAN WOMEN (ng/kg; fat basis)

	QUÉBEC (n=50)			ARCTIC (n=109)		
	n**	Mean***	CI 95%	n**	Mean**	CI 95%
DDE	572*	0.336	0.317-0.354	109	1.21	1.04-1.38
HCB	48	0.030	0.026-0.035	109	0.136	0.177-0.155
Heptachlor Epoxide	29	0.011	0.009-0.013	45	0.026	0.020-0.032
α chlordane				32	0.060	0.044-0.076
δ chlordane	0			18	0.010	0.007-0.011
Dieldrin	46	0.011	0.010-0.012	102	0.043	0.036-0.050
Endrin	0			1	0.008	- -
Mirex	3	0.008	0.004-0.012	90	0.0205	0.0163-0.0246

* From an on-going provincial survey (1989-1990)
** n= number of milk samples with detected concentration
*** Arithmetic mean

ORGANOCHLORINES AND HEAVY METALS IN THE BERING/CHUKCHI SEA ECOSYSTEMS, C.P. Rice, S.M. Chernyak, D. Hinckley, A. Krynitsky and T. Kolobova, Patuxent Wildlife Research Center, Laurel, Maryland, and Institute of Global Climate and Ecology, Moscow, USSR, and EA Engineering and Technology Incorporated, Hunt Valley, Maryland.

The distribution of organochlorines (OCs) and heavy metals in the Bering/Chukchi sea ecosystem is of interest for a number of reasons. Knowledge of their presence here is an indication that even pristine and remote areas of the environment are becoming contaminated with man-made pollutants and this information may allow early warning about possible toxics problems in these areas. Also the Bering/Chukchi area is unique for its high productivity and toxicity problems may cause extensive disruptions. Up to now there have been no comprehensive research studies made in the Bering and Chukchi Seas by the US and USSR because of the political boundaries which existed there. There were many gaps in our knowledge about the critical processes which impact this region. During our joint US/USSR expedition to this area in 1988 we were able to extensively sample pattern this region. This allowed us to define patterns of OC and heavy metal distribution which were correlated to processes such as water temperature, shelf depth, ocean currents, air patterns and biological diversity. Some of these features will be dealt with in this paper. Data were gathered on the levels of organochlorines in the air, water, surface microlayer, biota (zooplankton, phytoplankton, neuston, benthos and fishes) and sediment in these ecosystems. Metals were studied in the biota, water column and sediment.

The observations of Ocs in the air confirmed observations from other Arctic regions (Patton et al. 1989) that the hexachlorocyclohexanes (esp. alpha- and gamma-) are a major component of the overall level of organochlorines in the air over this region. Constituting more than 80 % of the total which was distributed as follows: 317 pg/m^3 total hexachlorocyclohexanes, 9.4 pg/m^3 total chlordanes and 38 pg/m^3 toxaphenes (PCB and total DDT residues were invalidated by shipboard contamination). In the surface water a similar ratio of distribution of the organochlorine class of compounds was observed (average of 34 observations) (3.4 ng/L total HCHs, 0.014 ng/L total chlordanes, 0.44 ng/L PCB and 0.01 ng/L total DDT residues) indicating that there was an equilibrium transfer process taking place between the air and water of this system. At deeper water levels the distribution of components begins to vary with the hexachlorocyclohexanes, especially, becoming less important to the total. This likely reflects the fact that sedimentation and revolatilization out of the upper layers of the water column may be taking place as well as degradation and other process of selective removal. Also their were some difference in the levels of components in the air, especially the HCHs, depending on the locations where the samples were taken and how the winds were moving. For the HCH isomers, there was also a suggestion from the surface water data that the relative levels varied with the degree of latitude a feature which has been noted by other scientists at least on a global scale (Calamari et al. 1991).

The biota in the water column exhibited bioaccumulation patterns which reflect the physicochemical properties of the individual pollutants. For example the HCHs were accumulated to a lesser extent than the chlordane isomer, trans nonachlor. Trans nonachlor has an octanol water partition coefficient (KOW) of 6.1; whereas, alpha HCH has an KOW of

4.0. The lower coefficient for alpha HCH reflects its greater water solubility relative to the chlordanes.

The proportion of these organochlorine components in the sediment of the Bering/Chukchi sea ecosystems indicated that accumulation is steadily occurring in the bottom sediments; however their uniformity over the entire area suggests that the materials are not building up in any high deposition basins and that the materials are becoming evenly distributed indicating a long period of recycling which allows these equilibria to take place. Considering the high productivity in the shelf area, there is probably an excess of particulate material to scavenge the relatively low level of pollutants which exists here. Therefore the sediment-water interface in the shelf area probably has little likelihood that high interface concentrations of toxic components ever occur.

Toxaphene residues were found in several compartments of these ecosystems including air, sediment, and biota (Table 1). In fact toxaphenes were second highest in concentration to the HCH group in the air samples and they were the highest of the OC residues measured in the two fish samples which were checked. The toxaphenes were analyzed using negative chemical ionization mass spectrometry. This is a technique that provides both compound verification and excellent sensitivity for this often difficult- to-analyze compound.

TABLE 1

CONCENTRATION OF TOXAPHENES IN SELECTED SAMPLES

SAMPLE	CONCENTRATION (NG/G WET WT.)	NUMBER OF RETENTION TIME MATCHED PEAKS*
POLLACK	10.80	29.00
POLLACK	(10)**	20.00
NEUSTON	(4)**	15.00
HERMIT CRAB	(2)**	7.00
ZOOPLANKTON	(2)**	15.00
PHYTOPLANKTON	(1)**	6.00
SHRIMP	(1)**	6.00
SEDIMENT	(0.3)**	4.00
*There were a possible 68 peaks from the standard to be matched.		
**The parenthesis indicate that these are estimated concentrations.		

Heavy metals were measured in sediment, biota and the water column. Levels were representative of a "clean" system with low to non-detectable values at all sites. The relative concentrations of cadmium and arsenic were slightly elevated in some of the benthos from the shelf areas; however, none were at levels which could be considered harmful.

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CHROMIUM (III) CONCENTRATIONS IN THE RECIPIENT OUTSIDE A TANNERY IN SOUTH GREENLAND.

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ABSTRACT

The tannery is situated in the village Qaqortoq in South Greenland. Chromium (III) is used in the tanning process and discharged without treatment directly to a 300 m deep fiord. About 600 kg chromium (III) is discharged per year. In October 1989 samples of Blue mussel, seaweed, fish, sediment, and sea water were collected and analysed. A village 25 km away served as reference area. The highest chromium (III) concentrations found in Blue mussel (4.9 µg/g d.w.) and seaweed (8.9 µg/g d.w.) were 10 and 55 times higher than reference values, respectively. More than 1 km away only low concentrations were found. No elevated concentrations were found in fish, sediment, and sea water. Acute toxicity to salt water invertebrates can be expected in the discharge area, whereas data on cronic toxicity of chromium (III) to salt water organisms are not accessible. The chromium (III) concentration found in Blue mussel and seaweed has been built up in less than one year, and still higher concentrations can be expected. Therefore, a renewed investigation is carried out on Blue mussel, seaweed, and scuplin (liver) in 1991.

INTRODUCTION

On behalf of the Greenland Home Rule the Greenland Environmental Research Institute in October 1989 conducted an investigation of the chromium (III) outlet from the Tannery of Greenlan(Glahder 1990). The tannery is situated in the village Qaqortoq in South Greenland (figure 1).

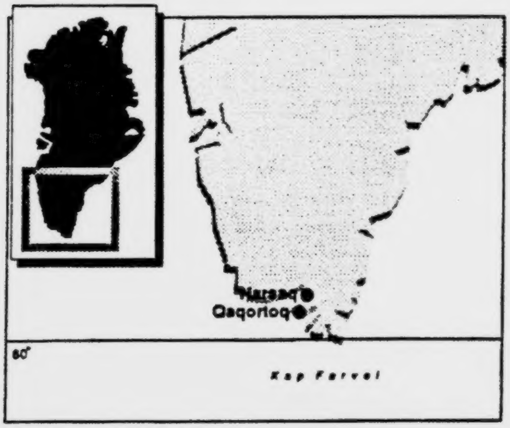


Figure 1. The situation of Qaqortoq in South Greenland.

The purpose of the investigation was to calculate the total amount of chromium (III) discharged per year, assess chromium (III) - concentrations in the environment and evaluate these concentrations with a reference situation.

The tannery in the late 1970'es used aluminium in the tanning process, but changed to chromium in 1988. The waste water is without any treatment discharged at the outer harbour directly to the 300 m deep Qaqortoq fiord (figure 2 and 3).

MATERIAL AND METHODS

During the first week of October 1989 samples were collected in the harbour and fiord of Qaqortoq and in the harbour and sound of Narsaq some 25 km away, the latter serving as a reference area. At a total of 47 stations 435 samples were collected: 12 stations with seaweed (Fucus vesiculosus) and Blue mussel (Mytilus edulis), 6 stations with Atlantic cod (Gadus morhua), Greenland cod (Gadus ogac), Shorthorn sculpin (Myoxocephalus scorpius) and Greenland halibut (Reinhardtius hippoglossoides), 25 stations with sediment and 4 stations with water samples.

To avoid contamination of the samples, growth tips of seaweed were cliped with a pair of white plastic scissors, soft parts of Blue mussel were cut with chromium-free scalpels, fish were cleaned with a titanium knife, and the periferi of the sediment column was removed with a white plastic knife.

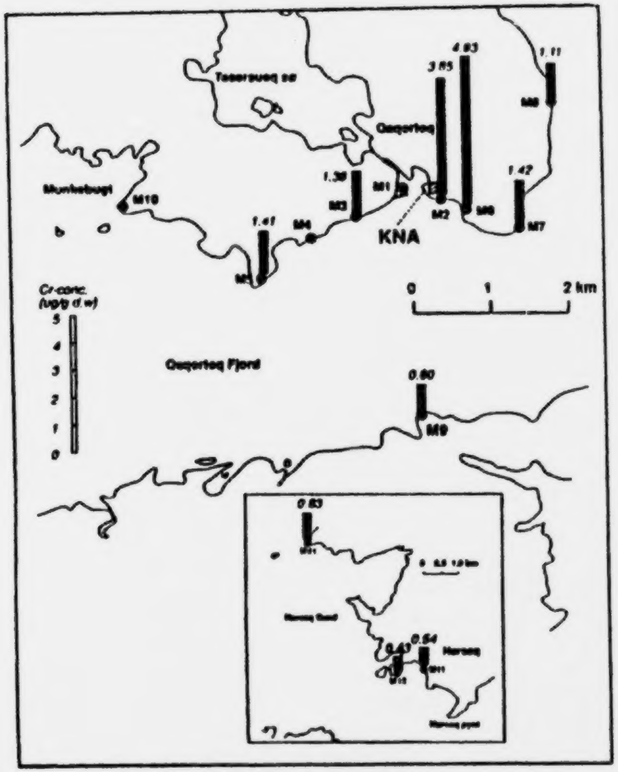
Samples of waste water were collected from vessels and drums as the tanning process is performed batchwise. A total of 86 representative samples were collected.

Seaweed, Blue mussel, and sediment were freeze-dried and homogenized and samples of muscle and liver from fish were cut with a titanium knife. These and waste water samples were dissolved in nitric acid using teflon bombs, and chromium concentrations were determined using flame or graphite furnace AAS and standard addition. Results are expressed on a dry weight basis. Sea water samples were analysed by cathodic stripping voltammetry using the differential puls mode and standard addition (Golimowski 1985).

RESULTS

Blue mussel. At each station three size groups of Blue mussel were analysed and it was examined if the chromium (III) concentration was a function of the soft part, shell weight and shell length, respectively. As this was not the case, results in table 1 and figure 2 are not corrected in this respect.

Close to the tannery concentrations in Blue mussel are 4 to 5 μg/g d.w., being significantly different from both mussels 1 to 3 km from the tannery (concentrations app. 1.5 μg/g d.w.) and from the mussels in the reference area at Narsaq (app. 0.5 μg/g d.w.); the two latter groups also differs significantly (according to the Tukey-test). Seaweed. Two or three samples from each station have been analysed and the result is shown in table 1 and figure 3. Chromium concentrations at the three stations closest to the tannery are significantly different from each other, and concentrations at these three stations are significantly higher than at all other stations, i.e. elevated 7,20 and 55 times, respectively.


Tasersuaq sø
Qaqortoq
Munkebugt
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4.93
1.11
M8
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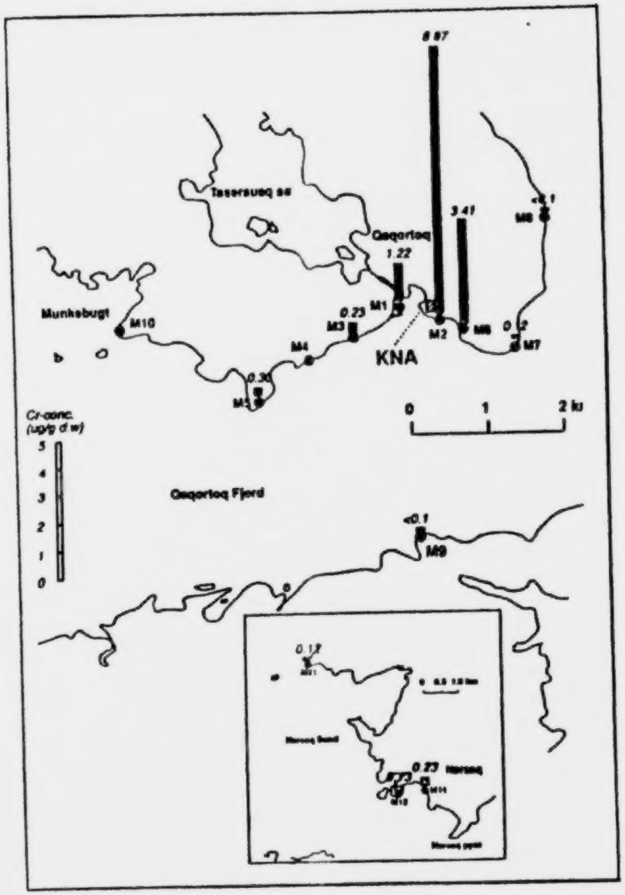
M3
M1
M2
M6
1.42
M7
1.41
M4
M5
KNA
Cr-conc.
(ug/g d.w)
5
4
3
2
1
0
0
1
2 km
Qaqortoq Fjord
0.80
M9
0.83
M11
0 0.5 1.0 km
Narsaq Sund
0.43
0.54
Narsaq
M12
M11

Figure 2. Chromium (III) concentrations (μg/g dry weight) in Blue mussel (Mytilus edulis) at Qaqortoq (KNA = Tannery of Greenland) and Narsaq.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Qaqortoq
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Munkebugt
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Figure 3. Chromium (III) concentrations (μg/g d.w.) in seaweed (Fucus vesiculosus) at Qaqortoq (KNA = Tannery of Greenland) and Narsaq.

Table 1 Chromium (III) concentrations ($\mu g/g$ dry weight) in Blue mussel (*Mytilus edulis*) and seaweed (*Fucus vesiculosus*) at Qaqortoq and Narsaq (s.d. = relative standard deviation).

Station no.	Blue mussel		Seaveed	
	geometric mean	s.d.	geometric mean	single sample
Qaqortoq				
M2	3.85	3.52-4.21	8.87	8.57;9.19
M6	4.93	4.87-4.99	3.41	3.62;3.21
M7	1.41	1.29-1.54	0.123	0.51;<0.1
M8	1.11	1.02-1.20	<0.1	<0.1;<0.1
M1	-	-	1.22	0.776;1.62
M3	1.38	1.28-1.49	0.300	0.321;0.280
M4	-	-	-	-
M5	1.41	1.22-1.63	0.234	0.213;0.257
M10	-	-	-	-
M9	0.79	0.75-0.83	<0.1	<0.1;<0.1;0.1
Narsaq				
M11	0.54	0.45-0.65	0.228	0.263;0.197
M12	0.43	0.34-0.55	0.129	0.167;<0.1
M21	0.79	0.60-1.05	0.115	0.151;<0.1;<0.1

Fish. Chromium was neither discovered in muscle nor liver in any of the analysed fish species, i.e. concentrations were below the detection limit of 0.1 $\mu g/g$ d.w.
Sediment. Chromium concentrations in sediments from different stations and at depths from 0 to 18 cm did not differ significantly. The range was between 16-45 $\mu g/g$ d.w.
Sea water. Chromium concentrations in sea water at four stations and at depths from 2 to 10 m (one down to 250 m) were 151 ± 31 ng/l, a little less than the chromium concentrations in non-polluted Atlantic sea water at 184 ± 16 ng/l (Berman 1982).
Waste water. A total discharge of about 600 kg chromium (III) in 1990 has been estimated on basis of chromium concentrations in the different batches. As the estimated amount of chromium used in the tanning process is 1053 kg, 57% of chromium is discharged. In 1989 650 kg chromium was used with a calculated discharge of 370 kg chromium.

DISCUSSION

When sampling took place in October 1989 about 370 kg of chromium (III) were discharged from the tannery. Prior to 1989 no chromium (III) has been discharged from this production. Waste water is discharged at the eastern coast line of Qaqortoq with a relative high temperature (mean: 21°C) so chromium will stay in the surface at first. Later chromium, mostly particle-bound, will start to settle as temperature decreases and because of an often high salinity of the waste water. The surface currents will move the chromium along the coast shifting from east to west with the tide (Grønlands Tekniske Organisation 1970). These conditions can explain why the highest chromium concentrations in seaweed and Blue mussel are found within 1 km from the tannery and at the eastern coast line (see figure 2 and 3). Chromium concentrations in Blue mussel show "medium" levels at distances between 1 and 3 km's from the tannery, indicating chromium still present in the surface water along the coast at each directions, while only little chromium seems to cross the Qaqortoq fiord (figure 2, station M9). Interesting, these "medium" levels are not found in seaweed, where concentrations outside 1 km are at the "reference" level. Reference values in South Greenland for Blue mussel (app. 0.5 μg/g d.w.) and seaweed (app. 0.2 μg/g d.w.) are low compared to relatively unpolluted sea waters in Denmark (Mogensen 1978), England and Sweden (Balsberg-Påhlsson 1982), being 1 to 3 μg/g d.w. for Blue mussel and 2 to 4 μg/g d.w. for seaweed.

Chromium (III) concentrations in mussels can in heavily polluted waters reach much higher values than 4.93 μg/g d.w. (Blue mussel at Qaqortoq). 5,000 μg/g w.w. were found in Blue mussels 20 m's from a wreck leaching app. 1 ton chromium (III) and a year later the concentrations were up to 60 μg/g d.w. 100 m's from the wreck (Balsberg-Påhlsson 1982). Outside a tannery in Denmark with a yearly discharge of 22 tons chromium (III) Blue mussel 400 m's away contained 51 μg/g d.w. and the mussel Macoma baltica contained 218 μg/g d.w. (Mogensen 1978).

Chromium concentrations in fish were all below the detection limit (0.1 μg/g d.w.), which was expected. In polluted waters higher concentrations can be found, especially in gills, but also in kidney and liver (Balsberg-Påhlsson 1982).

Sediment concentrations in the harbour of Qaqortoq were low and at the same level as found in non-polluted areas in Sweden, between 20 and 40 μg/g d.w. (Balsberg-Påhlsson 1982). This indicates that non or only little sedimentation takes place in the harbour due to the heavy tide.

As the tanning process is performed batchwise, each batch of 2-3,000 l, contains very different chromium (III) concentrations with highest concentrations, between 1-1.5 g/l, found in the tanning batches after tanning (Jensen 1990). One or two of these batches are discharged every day. As acute chromium (III) values for the salt water species American oyster (Crassostrea virginica) and zoealarvae of a crab (Sosarma haematocholor) are 10.3 and 56 mg/l (Ambient Water Quality Criteria for Chromium 1980), acute toxicity can be expected in the discharge area, but no tests have yet been performed. No data on cronic toxicity of chromium (III) to salt water organisms have been found.

If a person eats 50 Blue mussels with the highest chromium (III) concentration this will give the person a daily intake of 175 μg which is the upper part of normal chromium intake and three times the daily need for chromium, but still much less than the body can tolerate (Balsberg-Påhlsson 1982). Neither a Danish acceptable daily intake (ADI) nor a WHO provisionally tolerable weekly intake (PTWI) for chromium is established (Alsing 1990).

CONCLUSION

Chromium concentrations in Blue mussel and seaweed has in less than one year been elevated 10 and 55 times, respectively, and as chromium discharge in 1990 has raised, even higher concentrations can be expected.

No chromium has been found in fish, and no hazard to human health is likely.

Acute toxicity to salt water invertebrates can be expected in the discharge area.

It is recommended to monitor the chromium concentration in Blue mussel, seaweed, and sculpin (liver, gill) every 2. or 3. year, control the chromium discharge, perform acute toxicity tests on invertebrates and optimize the tanning process.

The monitoring and discharge program are carried out October 1991, and optimizing the tanning process has started earlier in 1991.

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HEAVY METALS AND SELENIUM IN ARCTIC MARINE MAMMALS.

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Marine mammals are harvested for food and income throughout Arctic Canada. Marine mammals are also at the top of the trophic web making them likely repositories of contaminants. These two characteristics create a need to measure and understand the levels of contaminants in these animals to build toward assessing the impact of contamination on the populations of seals, whales and walrus and on the populations of humans which depend upon them. Here we present new data on tissue levels of six metals in three tissues of walrus from three Arctic locations and evaluate them with respect to earlier data for other marine mammals.

Walrus were sampled in conjunction with Inuit harvests at Igloolik, Hall Beach and Iqaluit, Northwest Territories in the summers of 1982-1984, and 1987-1988. Samples of liver, kidney and muscle were kept frozen until analyzed for lead, cadmium, zinc, mercury, copper, and selenium following Wagemann et al. (1983). Ages were determined by blind replicate counts (Stewart and Lavigne 1979) of cementum layers in thin longitudinal sections of a lower canine tooth (Garlich-Miller et al. submitted). Associations among metals were examined by principle components analysis (PCA), multiple analysis of variance (MANOVA), and robust multiple stepwise regressions to assess effects of age and location. There were too few females sampled at Hall Beach and Iqaluit to test between genders so further discussion is limited to males.

Selenium levels varied with age at all three locations, as did cadmium at Igloolik and mercury and copper at Iqaluit. However the distribution of ages in the samples did not differ significantly among sites ($X^2 = 8.65$, 8 d.f., $P > 0.25$) and means were analysed. Concentrations of lead, zinc, and cadmium were independent of age. For males, none of the mean concentrations measured differed among locations.

Overall mean concentrations (Table 1) of mercury were low in all three tissues compared to beluga (Delphinapterus leucas) (Wagemann et al. 1990) and ringed seals (Phoca hispida) (Wagemann and Muir 1984). Lead was relatively high in all tissues and cadmium was high in liver and kidney.

The source of high levels of metals in some Arctic marine mammals remains unknown. Narwhal sampled near a lead-zinc mine in north Baffin Island had elevated levels of cadmium (Wagemann et al. 1983) which may be from natural sources or anthropogenic sources in Canada or Greenland. Commercially viable deposits have not been found near Igloolik or Hall Beach. The underlying rocks there are the type which contain lead, zinc, and cadmium (Thorsteinsson and Tozer, 1970), but the naturally occurring levels in these rocks are usually an order of magnitude lower than commercial deposits. However, walrus feed heavily on bivalve molluscs (Fisher 1989) which are known to accumulate heavy metals (Fallis 1982). Clams removed from the stomachs of walrus in our sample had lead levels around 0.20 ug/g wet mass. Our preliminary analysis suggests a need for more detailed study of the sources - natural or not - of heavy metals related to the concentrations of these metals found in marine mammals.

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Table 1. Overall mean concentrations and standard deviations (μg/g dry wt) of metals in tissues of male walrus (*Odobenus rosmarus rosmarus*) from Igloolik, Hall Beach and Iqaluit.

	LIVER	KIDNEY	MUSCLE
Pb	0.28±0.23	no data	0.067±0.096
Cd	35.5±18.7	235±107	0.50±0.46
Zn	146±32	164±31	178±29
Hg	4.84±4.04	1.47±0.52	0.30±0.25
Cu	35.4±27.3	21.7±6.41	3.12±0.79
Se	9.06±3.26	no data	11.1±3.8

RECENT STUDIES ON HEAVY METALS IN POLAR BEARS FROM GREENLAND WITH REFERENCE TO OTHER MARINE MAMMALS.

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Extended abstract:

Concentration ranges

Muscle, liver, and kidney tissue from 38 polar bears caught in the Scoresby Sound, central east Greenland, were analysed for zinc, cadmium, mercury, and selenium. The results of the present study are presented in Table 1. Lentfer and Galster (1987) previously published results on mercury in Alaskan polar bear muscle and liver. The mercury levels in muscle and liver of polar bears from east Greenland were intermediate to levels from western and northern Alaska. Norstrom *et al.* (1986) analyzed livers of 67 polar bears from Canada for 22 elements. This investigation *showed* no geographical differences in zinc concentrations, and in concordance with this the zinc level in liver of polar bears from east Greenland was equivalent to that of canadian bears. Norstom *et al.* (1986) found significantly higher cadmium levels in livers of polar bears from the eastern than from the western Canada. This esta-west trend can be extended to east Greenland as well.The concentration vs. age regression line from this east Canada and the corresponding line for the east Greenland polar bears are presented in Figure 1. As seen from the figure the cadmium level is approximately 0.4 µg/g higher in east Greenland, but the rate of accumulation seems to be the same in the two areas. Regression equations for mercury versus age were presented for two areas of Canada by Norstrom *et al.* (1986). A comparison of these regression lines is given at Figure 2. The geographical differences are much more pronounced than for cadmium and show the opposite geographic trend. Hence polar bears from central and northeast Canada accumulated mercury four times faster than bears from east Greenland and the bears from northwest Canada accumulated mercury 14 times faster. The geographical differences within Canada have been linked to differences in the composition of the polar bear diet. Hence Eaton and Farant (1982) and Norstrom (1986) suggest the higher percentage of bearded seals relative to ringed seals in the polar bear diet of western Canada compared to eastern Canada to explain the differences. This explanation can hardly cover the extended trend towards east Greenland as well. Also, the decreasing trend towards east for mercury in polar bear hair have been observed by Renzoni and Norstrom (1990) and Born *et al.* (in prep.) to cover Svalbard as well. The interest in bearded seals versus ringed seals is based on data on mercury in liver of the two species, concentrations in bearded seals being five times higher than in ringed seals (Smith and Armstrong 1978). However, whether the differences exists in blubber as well and whether ringed seals hold higher cadmium levels than bearded seals, to explain the opposite geographical trend for this metal, remain undocumented. In order to test the hypothesis of Norstrom *et al.* (1986), figures from the Greenland Environmental Research Institute (GERI) database on mercury and cadmium in blubber, muscle, liver, and kidney were compared for ringed and bearded seals from the Thule district, northwest Greenland. Bearded seals had significantly higher mercury levels in liver than ringed seals ($P < 0.01$; $t = -2.783$, $DF = 36$), in concordance with the finding of Smith and Armstrong (1978), however, no other tissue showed a significant difference for mercury, and the cadmium levels in all four tissues were the same in the two seal species. Therefore, the theory of differing bear diet composition regarding ringed and bearded seals as an explanation for the geographical trend of mercury and cadmium in polar bear can not be confirmed.

Ringed seal is by far the most important food item of the polar bears (*e.g.* Lønø 1970, Stirling and McEwan 1975, Stirling and Archibal 1977, Furnell and Oolooyuk 1980, Smith 1980, Best 1987). The heavy metal contents of ringed seals from the same geographical area and period were therefore compared to the polar bear levels. The polar bear muscle level of the essential metal zinc was signifi-

Table 1. Element concentrations found in polar bears. Geometric means (GM), ranges, and number of observations (N). All values are µg/g wet weight.

	Muscle				Liver				Kidney			
	Min	GM	Max	N	Min	GM	Max	N	Min	GM	Max	N
Zinc												
Juvenile	70.2	70.2	70.2	1	35.8	37.7	39.0	3	18.2	19.7	20.7	3
Subadult	53.3	59.3	70.2	12	32.6	56.5	84.1	20	19.1	33.8	52.9	20
Adult	50.3	62.7	80.4	5	32.3	49.8	95.2	15	25.2	40.5	77.6	15
All	50.3	60.8	80.4	18	32.3	52.1	95.2	38	18.2	34.8	77.6	38
Cadmium												
Juvenile	0.019	0.019	0.019	1	0.203	0.233	0.254	3	1.04	2.17	3.60	3
Subadult	<0.015	0.016	0.038	12	0.092	0.897	2.43	20	1.25	10.8	33.8	20
Adult	0.038	0.048	0.085	5	0.102	0.998	3.29	15	3.93	24.4	115	15
All	<0.015	0.022	0.085	18	0.092	0.841	3.29	38	1.04	13.1	115	38
Mercury												
Juvenile	0.101	0.101	0.101	1	1.35	1.81	2.84	3	1.59	2.87	3.97	3
Subadult	0.039	0.065	0.193	12	2.51	7.42	14.6	20	4.90	11.5	45.8	20
Adult	0.045	0.082	0.141	5	3.19	11.4	24.8	15	13.8	30.8	66.6	15
All	0.039	0.071	0.193	18	1.35	7.87	24.8	38	1.59	15.2	66.6	38
Selenium												
Juvenile	-	-	-	0	0.670	1.09	1.72	3	1.59	2.34	4.29	3
Subadult	0.200	0.313	0.570	12	1.73	3.26	5.43	20	2.90	6.25	23.1	20
Adult	<0.200	0.240	0.420	5	1.41	4.54	8.24	15	1.53	6.63	24.4	15
All	<0.200	0.289	0.570	17	0.670	3.40	8.24	38	1.53	5.92	24.4	38

cantly (GM(polar bear)/GM(ringed seal) = R = 2.7; P < 0.0001 unpaired two-tailed t-test) higher than in ringed seal. The liver levels were not different (R = 1.01; P = 0.839), while the kidney level was significantly lower (R = 0.67; P < 0.0001) in the polar bear than in ringed seal. The cadmium levels were significantly (P < 0.0001) lower in polar bear than in ringed seal for all three tissues (muscle: R = 0.29; liver: R = 0.073; kidney: R = 0.343). These low cadmium levels may be explained by the preference of the bears for eating skin and blubber, a feeding behaviour that has been documented by a number of authors (e.g. Smith 1980, Stirling 1974, Stirling and Archibald 1977, Stirling and McEwan 1975). Norstrom *et al.* (1986) suggested this relationship based on low cadmium levels in their liver analyses. Johansen *et al.* (1980) showed that almost no cadmium is contained in seal blubber, mean blubber values of cadmium in ringed seals from Umanak and Upernavik, west Greenland were below the detection limit of 0.02 µg/g. In the same study ratios of blubber relative to muscle, liver and kidney were reported to be 1:5:500:2000. Mercury was significantly lower in muscle of polar bear compared to ringed seal (R = 0.27; P < 0.0001), while the opposite was the case for liver and kidney (liver: R = 1.99; kidney: R = 11.6; P< 0.0001). No data have yet been published for mercury in blubber of ringed seals of the Arctic, but figures from the GERI database on ringed seals from Thule, northwest Greenland showed geometric mean of 0.19 µg/g (range: 0.05-1.06 µg/g, n = 33). Median ratios of blubber relative to muscle, liver and kidney were approximately 1:1:80:400. Previously published levels from Sergeant and Armstrong (1972) showed that harbour, grey, hooded and harp seals from the eastern Canada had blubber levels of 0.03 - 0.08 µg/g, with average ratios relative to blubber of 1:15:40:500 for muscle, kidney and liver respectively. According to Siegstad (1988) the most dominant food item of the ringed seal i Thule is arctic cod (*Boreogadus saida*). Mean (geometric) mercury levels in muscle of arctic cod from Thule was 0.020 µg/g (range: <0.005 - 0.053 µg/g, n = 67). It therefore seems that the mercury concentration in the polar bear diet is a factor 10 higher than in the diet of the ringed seal. For neither muscle nor liver significant differences in content of selenium could be observed (muscle: R = 1.12, P = 0.545; liver: R = 0.84, P = 0.448). Finally, the kidney level of selenium was significantly higher (R = 2.38; P < 0.0001) in polar bear than in ringed seal. No results on heavy metals have previously been published for kidney from polar bears, hence no comparisons can be made with our results.

Correlations of element concentrations with sex and age

No significant sex differences were found for either zinc, cadmium, mercury or selenium in any of the three tissues analyzed. This is in concordance with Renzoni and Norstrom (1990) and Born *et al.* (in prep) who found no significant differences between sexes in polar bear hair. Norstrom *et al.* (1986) excluded sex from analysis due to non-significant effect for polar bear liver.

A negative correlation of zinc in muscle with age is consistent with findings on humans, where the requirement of zinc in young in connection with growth is larger than for grown-ups (Cousins 1985; Elinder 1986). In concordance with Norstrom *et al.* (1986) no age relation could be found for zinc in liver. A positive correlation with age in kidneys might be ascribed to a co-accumulation with cadmium. It has been well documented on a number of animals that cadmium induces the synthesis of metallothionein (Mt) (Andrews 1990). Other investigations show that cadmium and zinc are bound in a ratio 5:2 to Mt (Bremmer 1987), which becomes evident, beyond the essential zinc level, in high cadmium exposed tissue as kidney.

Correlations between age and concentration of cadmium and mercury were highly significant in liver and kidney. Norstrom *et al.* (1986) also found a significant age accumulation for certain Canadian areas in liver of polar bear (see comparisons Fig.1 and 2). A lack of significance for these two metals in muscle might be ascribable to an efficient clean-up of cadmium and mercury by the liver resulting in low levels in the muscle (close to the detection limit). Another factor could be the smaller number

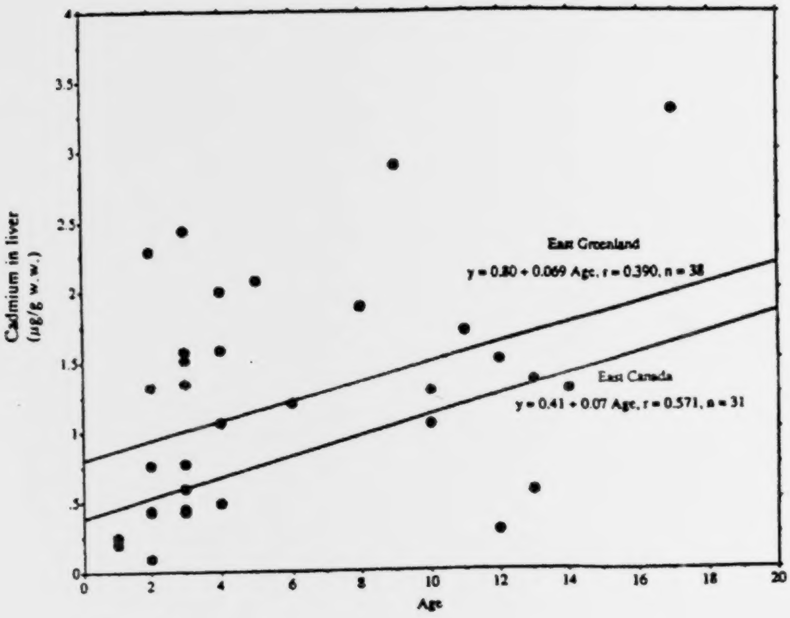

Figure 1. Cadmium in polar bear liver versus age from east Greenland. The regression line calculated from these observations is plotted and so is the corresponding regression from east Canada (Norstrom et al. 1986).

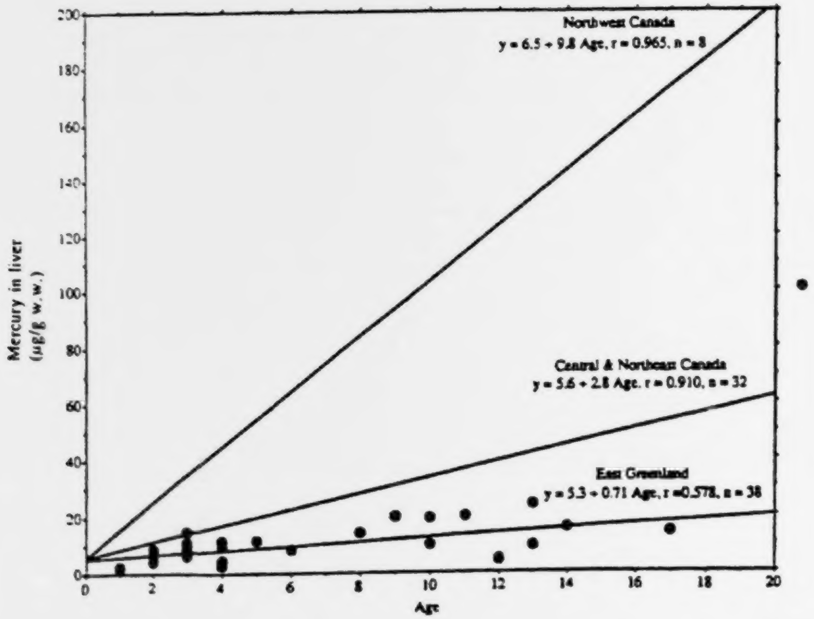

Figure 2. Mercury in polar bear liver versus age from east Greenland. The regression line calculated from these observations is plotted and so is the corresponding regression from central & northeast Canada and northwest Canada (Norstrom et al. 1986).

of analyses carried out on muscle tissue.

Selenium was only correlated with age in liver tissue. Norstom *et al.* also found a significant trend with age and geographical regions for selenium in polar bear liver, as could be expected by the high correlation of mercury and selenium.

Inter- and intra-organ differences and correlations

The finding of different metal levels in the different tissues is in concordance with previous findings for marine mammals (e.g. Smith and Armstrong 1978, Dietz *et al.* 1990, Hansen *et al.* 1990). The positive correlations found between liver and kidney for all metals possibly reflect age accumulation of the metals in the two organs despite a few missing correlations between zinc in liver and selenium in kidney related to age. The negative correlation between muscle and liver found for zinc could be explained in the same way, since zinc was negatively correlated with age in muscle. The lack of correlations for muscle interactions were probably linked to low N as stated by Nielsen and Dietz (1989).

The organ that contains the highest mercury levels is the kidney in polar bear, as in terrestrial mammals in general (WHO 1976). For other marine mammals the liver holds the highest mercury levels (e.g. Hansen *et al.* 1990, Julshamn *et al.* 1987). This difference is likely to be caused by the high content of selenium in the food items of marine mammals in general. Selenium has an effect on the inter-tissue distribution of mercury in the organism, thus Cirkt and Bencko (1989) found that exposure of selenium caused an decrease in the kidney and an increase in the liver, brain and blood.

Mercury and selenium showed molar ratios close to 1:1 in liver and kidney. This finding has previously been shown for marine mammals by e.g. Koeman *et al.* (1973; 1975), Hansen *et al.* (1990), and Nielsen and Dietz (1990). Koeman *et al.* (1975) suggested that selenium was involved in a specific chemical mechanism being able to detoxify methyl mercury. Hansen *et al.* (1990) noted a tendency in livers with mercury levels below 8 µg/g (corresponding to app. 40 nmol/g) to have molar ratios of Hg:Se below unity, explained by the essential demands for selenium. The same picture appeared for polar bear liver where a close 1:1 ratio was found above 30 nmol/g. If a selenium and organic mercury enters a less toxic complex in the excess of app. 6-8 µg/g, one could expect a cease in the organic mercury accumulation around this concentration. This relation was shown in livers of Greenland marine mammals by Dietz *et al.* (1990), where organic mercury did not exceed 2.2 µg/g even when total mercury was 50 times higher. Hansen *et al.* (1990) also presented figures on molar ratios of mercury and selenium in kidney and found an excess of selenium. The polar bear data in kidney were closer to unity, which could be explained by higher concentrations of mercury (above 40 nmol/g).

Also in polar bear kidney the cadmium-selenium ratio is around 1:1 in molar terms. Whether this is incidental, the co-accumulation of and selenium being caused by independant age accumulation of theelements (Hansen *et al.* 1990), or an effect of selenium acting against toxic cadmium as suggested by experiments on rats (e.g. Parizek *et al.* 1971), remains to be clarified.

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CONTAMINANTS IN NORTHERN QUÉBEC: LEVELS AND TRENDS.

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ABSTRACT: The presence of contaminants in northern regions is now a well known fact. Various studies have yielded the levels of several types of contaminants in northern Québec and Labrador. The particularly toxic metals (Hg, Cd & Pb) and organochlorines (PCB, DDT, dieldrin, chlordane & HCB) were chosen for this study. However data is sparse and few studies have been made but recently.

Through literature review and collection of unpublished data, a database was set up to manage a multitude of information. Using this last, we were able to assess the contamination levels available in organisms of the aquatic food chain of northern Québec. The study was limited to material sampled after 1979. For example, beluga blubber from the Hudson region was found to have concentrations of Σ PCB and Σ DDT of 2000 and 2500 ng/g (wet weight) respectively; while ringed seal blubber showed levels of 500 and 600 ng/g (wet weight) respectively. Hg has been extensively studied in fish due to hydroelectric projects while levels of other metals and organochlorines were neglected.

RÉSUMÉ: La présence de contaminants dans les régions nordiques est maintenant un fait reconnu. Diverses études ont rapporté les niveaux de plusieurs types de contaminant dans le nord du Québec et au Labrador. Les métaux (Hg, Cd & Pb) et les organochlorés (BPC, DDT, dieldrine, chlordane & HCB) choisis pour cette étude le sont pour leur toxicité particulière. Par contre les données sont plutôt rare et seule quelques études très récentes sont disponibles.

Une base de données a été élaborée à partir d'une revue de littérature et d' un regroupement de données non publiées afin de gérer une multitude d'information. Avec l'aide de cet outil, il a été possible d'évaluer les données disponibles sur les niveaux de contamination dans les organismes de la chaîne alimentaire aquatique du nord québécois. La présente étude a été limitée aux données échantillonnées après 1979. Par exemple, le gras sous-cutané du béluga de la région de l'Hudson a des concentrations de 2000 ng/g en Σ BPC et de 2500 ng/g (poids humide) en Σ DDT; tandis que le phoque annelé a des niveaux de 500 et 600 ng/g respectivement. Le Hg a été très étudié chez le poisson en raison des projets hydroélectriques tandis que les autres métaux et les organochlorés ont été négligés.

INTRODUCTION

Concerns about the state of arctic regions really begun but within the last decade. It also marked the steady increase of research dealing with the contamination condition of the arctic fauna. In view of results obtained, it did not take long before a certain uneasiness rose within the Inuit people whom depend greatly on country foods to survive in a harsh environment. Furthermore, hydroelectric projects which developed in northern regions showed signs of promoting mercury (Hg) accumulation in fish swimming in the waterways affected by terrain flooding.

In light of the possibility of human contamination through the aquatic food chain of the arctic, we decided to evaluate the contamination level of several contaminants which are known to be highly toxic. This study includes the Hudson region and the Ungava region as defined in Figure 1.

MATERIALS AND METHODS

A thorough review of published and unpublished data was conducted to find any data available on the

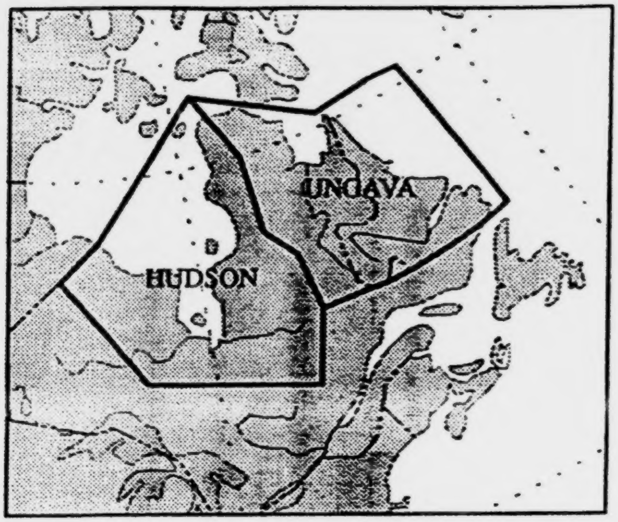


Figure 1. Delimitations of the Hudson and Ungava regions.

Table 1 **Means of mean concentrations of metals studied in aquatic animals of the Hudson and Ungava regions (ng/g, wet weight).**

Species		Hg	Hg (LGC)	Hg (CFC)	Cd	Pb
White Sucker	(1)*	219 (100)	-	-	32 (22)	71 (81)
	(2)	-	-	125 (62)	-	-
Longnose Sucker	(1)	164 (129)	543 (338)	-	-	-
	(2)	362 (178)	-	256 (230)	-	-
Lake Trout	(1)	741 (701)	1387 (816)	-	-	-
Brook Trout	(1)	223 (221)	829 (1035)	-	-	-
	(2)	90 (0)	503 (279)	128 (123)	-	-
Arctic Char	(1)	200 (80)	-	-	-	-
	(2)	39 (4)	-	-	-	-
Lake Whitefish	(1)	162 (99)	502 (381)	-	-	-
	(2)	140 (360)	335 (201)	188 (99)	-	-
Northern Pike	(1)	752 (510)	1642 (956)	-	-	-
	(2)	890 (-)	1140 (360)	675 (406)	-	-
Atlantic Salmon	(2)	40 (0)	-	-	-	-
Beluga †	(1)muscle	800 (530)	-	-	43(48)	50(90)
†	(1)liver	8310(10380)			4090 (2140)	37 (35)

N.B. Fish analyses were done on muscle unless otherwise stated;
Beluga analyses were on the liver and muscle
† : mean estimated from dry weight basis using conversion factors from *Wagemann et al.* (1991).
* analysis were done on the whole animal
(1): Hudson Region
(2): Ungava Region
(LGC): "La Grande Complex"
(CFC): "Churchill Falls Complex"

contamination of the aquatic food chain of the Canadian Arctic and Greenland. Based on the collected information, a database was setup using the 4th-Dimension software to maximize the possibilities of access. Any analysis of contaminant done on aquatic organisms located within our delimited regions were included for any future reference.

For this evaluation, only data sampled after 1979 was selected because of the increase in research starting in that period and also since techniques of analysis were more uniform by then. Fourteen contaminants were labelled as "highly toxic". The choice of tissue was limited to blubber, muscle, liver and whole animal in the case of fish samples; species were chosen in accordance to their priority in the diet of the native people. Research was lacking in many areas: be it for the contaminant itself or for the type of organism. Data was found for dieldrin, Σ PCB, Σ chlordane, Σ DDT, hexachlorobenzene (HCB), Hg, Cd and Pb; while no data was found for the TCDD, aldrin, lindane, endrin, heptachlor and methoxychlor.

Since data obtained from the database were already means of contamination levels in most case, a formula was used to statistically calculate the mean of the means for a specific contaminant, animal, tissu and region. It was therefore possible to incorporate various studies to generate a mean contamination level. This however implied that the methodologies of analysis used by researchers were consistent with one another.

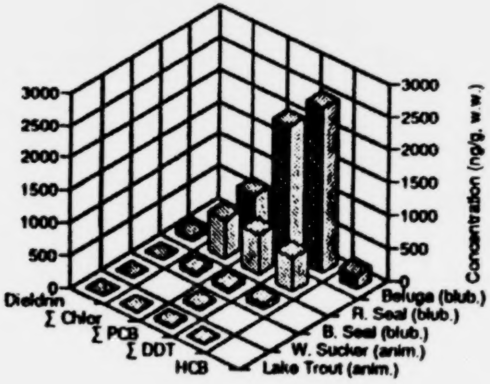
RESULTS

Mean concentrations were generated for each the Hudson and Ungava region. This choice was made to separate the hydrographic basins of the Hudson Bay and Ungava Bay. Mean concentrations of organochlorines are depicted in Figure 1, while mean metal concentrations are in Table 1. Hg levels within a hydroelectric complex and those found in a ¼natural¼ environment (i.e. where the rate of flow or level of water was not modified by manmade structures) were separated to permit a better interpretation of the data. Only those species for which data was found are listed. No data was available at this time on aquatic birds except for Hg in the muscle of one eider showing a geometric level of 50 ng/g (wet weight).

It is evident from Figure 2 that beluga is the most contaminated species by organochlorines, with blubber mean levels of Σ PCB, Σ DDT and Σ Chlor of 2000, 2534 and 1365 ng/g (wet weight) respectively. Ringed seal is somewhat less contaminated with levels of 614, 513 and 592 ng/g (wet weight) respectively, while bearded seal has lower levels than either. No data was available for walrus contamination by either organochlorine or metals. Little is known of organochlorine contamination in fish; White Sucker and Lake Trout are the only fish species with information on their contamination level at this time.

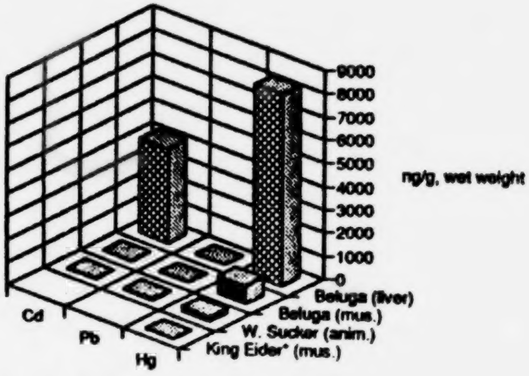
Studies in metal contamination were mostly directed toward fish and dealing almost solely with Hg contamination (see Table 1). This is due of course to the problem of increase of mercury bioavailability in newly made reservoirs within hydroelectric projects. Figure 4 illustrates differences in mercury contamination in fish with an environment affected by a hydroelectric complex and those in a ¼natural¼ environment. Metal levels in marine mammals were only studied in the beluga, with mean liver Hg levels of 8310 ng/g (wet weight), Cd levels of 4090 ng/g and Pb levels of 37 ng/g, and respective levels of 800, 43 and 50 ng/g (wet weight) in the muscle. Figure 3 gives a general representation of the differences of the contamination levels between sea mammals, fish and aquatic birds (no column means no data was available). One can note that the metal concentration in the liver of beluga are higher than those in the muscle. This is to be expected because of the tendency of metal contaminant to accumulate in the liver.

Figure 2. Levels of organochlorines in some animal species of northern Québec



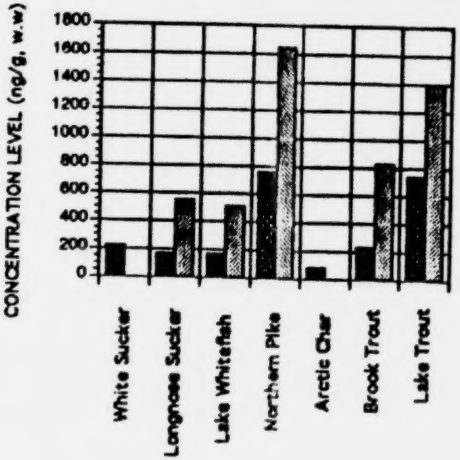
N.B. "N" varies from 5 to 28 individuals

Figure 3: Comparisons between classes of animals and their level of contamination in metals (ng/g, wet weight)



*Geometric mean

Figure 4. Mean concentrations of mercury in fish found in the Hudson region.



N.B. "N" varies between 18 and 2207 individuals
Dark columns are for "natural" environment, light for the La Grande Complex.

DISCUSSION

Eventhough contamination data of northern regions are still few, there is ample evidence of organochlorine and metal contamination in the aquatic food chain of northern Québec. Beluga whales, which are one of the top predators of the aquatic food chain, give a good indicator of an affected system. However one has to consider that there are species variation not only due to their food habits but also because of metabolic differences in dealing with an exposition to a contaminant. Values such as we have obtained could represent either an environmental problem, a problem within a species, or both.

Higher levels of Hg contamination within a hydroelectric complex are evident. But in this present study we can not account for the possible variations (be it increase or decrease) of levels in time. This also goes for any contaminant although it would be hard to determine since levels have to be generated periodically over at least a decade.

One thing which is certain is that more information is needed on the contamination level of the arctic fauna. Much data are lacking, especially in organochlorine contamination of fish and metal contamination in marine mammals. Levels would give an indicator of more southern problems since contaminants have a worldwide origin.

ACKNOWLEDGEMENTS

We would like to thank Hydro-Québec who helped to financially support the database project and gave us access to their data. We also wish to thank all who were good enough to give us access to unpublished data and databases, especially Derek Muir, who was always available for advices, and other personnels of the Freshwater Institute in Winnipeg (DFO). We are greatfull for the incredible cooperation of researchers and people dealing with the Arctic environment.

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SOURCES AND PATHWAYS OF ARCTIC CONTAMINANTS.

L.A. Barrie, Environment Canada, Atmospheric Environment Service, Downsview, Ontario.

EXTENDED SUMMARY

Potentially toxic organic compounds, acids, metals and radionuclides in the northern polar region are a matter of concern as it becomes evident that long-range transport of pollution on hemispheric to global scales is affecting this part of the world. In a recent review and assessment of sources, occurrence, history and pathways of these substances in the north(Barrie et al, 1991) the state of knowledge of the transport media--the ocean and atmospheric circulation--was examined. A five-compartment model of the northern region shows that we know most about pathways of acids, metals and radionuclides and least about those of complex synthetic organic compounds. Of the total annual inputs of anthropogenic acidic sulphur and the metals lead and cadmium to the Arctic via the atmosphere, an estimated 10 to 14% are deposited.

A water mass budget(Figure 1) for the surface compartment of the Arctic Ocean, the most biologically active part of that sea, is constructed to examine the mass budget(Table 1) for one of the major persistent organochlorine compound groups found in remote regions, hexachlorocyclohexanes(HCH), one isomer of which is lindane. It is concluded that both the atmosphere and the ocean are important transport media. Even for the HCH substances which are relatively easily measured and simple in composition compared to other synthetic organics, we know little about the occurrence and environmental physical/chemical characteristics that determine pathways into the food chain. More environmental measurements, chemical characterization studies and environmental chemical transport modelling are needed, as is better knowledge of the circulation of the Arctic Ocean and the marine food web.

As a result of this review and additional ones covering other aspects of the northern contaminants problem(Lockhart et al, 1991; Muir et al, 1991; Thomas et al, 1991) a 6 year Arctic research program on northern contaminants began in summer 1991. It will involve a host of pathways studies including measurements of occurrence of organochlorines in the Arctic atmosphere at several locations for two full years, development of estimates of global emissions of organochlorines and chemical transport models.

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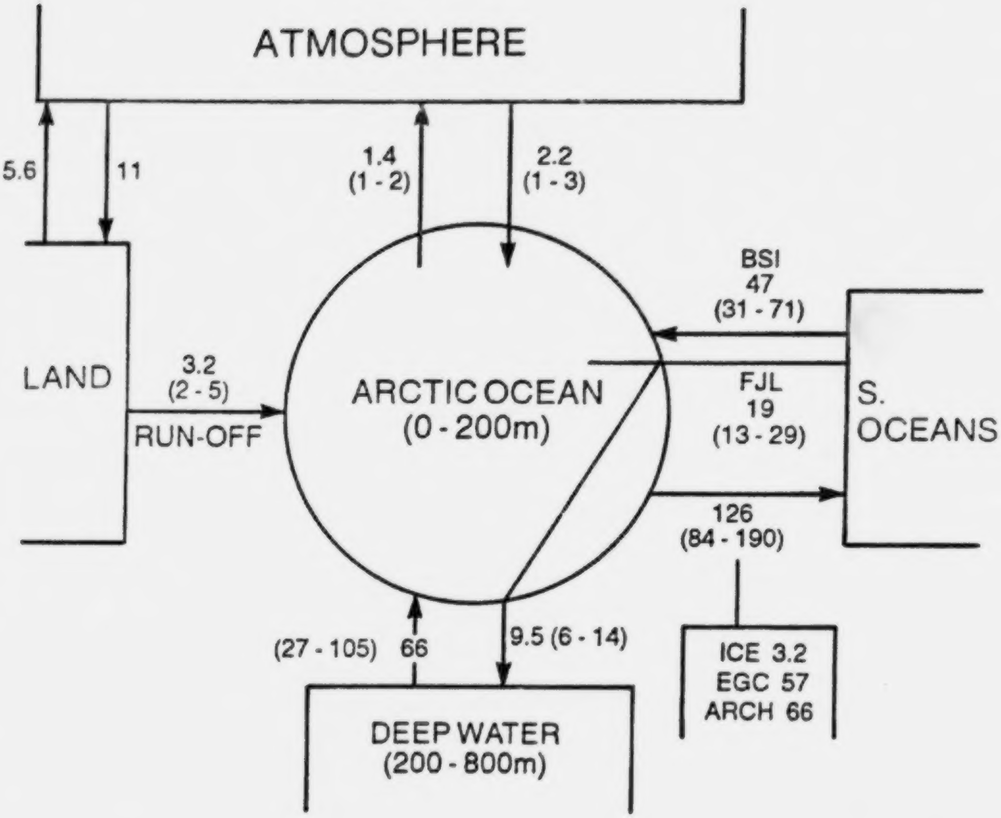
ATMOSPHERE
5.6
11
1.4
(1 - 2)
2.2
(1 - 3)
LAND
3.2
(2 - 5)
RUN-OFF
ARCTIC OCEAN
(0 - 200m)
BSI
47
(31 - 71)
FJL
19
(13 - 29)
S.
OCEANS
126
(84 - 190)
ICE 3.2
EGC 57
ARCH 66
(27 - 105) 66
9.5 (6 - 14)
DEEP WATER
(200 - 800m)

Figure 1 Arctic surface ocean water budget(Barrie et al, 1991) Annual flow of water by various pathways are given in 10^{12} m^3 of water.

FJL -net flow into Spitzbergen- Franz Josef Land- Novaya Zemlaya shelf area from North Atlantic. EGC - polar waters in the East Greenland Current. ARCH - flow from the Canadian archipelago to Baffin Bay. Note that the large volume flow rate West Spitzbergen current is contributing to the deep water layer as it enters and sinks below the ocean surface layer.

Table 1 Details of annual chemical budget estimates for alpha plus gamma hexachlorocyclohexane to the Arctic surface ocean(0-200m). For runoff and ocean mass flow estimates, the volume flow rates given in Figure 1 were used.

	Concentration C (ng l^{-1})	Estimated mass flow (tonnes y^{-1})	
Sources			
Atmospheric			
Wet & Dry Dep		16	(8-32)
Vapour Exchange		63	(16-252)
Ocean Currents			
Bering Strait	2.1-3.0	120	(66-212)
Deep Water	0.2-0.4	20	(9-39)
Franz-Josef-Land Current	0.9-1.5	29	(15-51)
Land			
Runoff	3.7-7.3	18	(8-35)
	Total Sources	**266(122-431)**	
Sinks			
Ocean Currents			
Ice	0.7-6.0	11	(2-29)
East Greenland Current	0.9-1.5	68	34-128)
Archipelago	2.0-8.0	330	(88-792)
Franz-Josef-Land Current	1.2-1.8	15	(5-20)
Chemical Transformation		unknown	
Sediments	not detectable	small	
	Total sinks	**424**	**(129-969)**

DEPOSITION AND FATE OF SEMI-VOLATILE TRACE ORGANICS IN ARCTIC SNOWPACKS.

D.J. Gregor, Environment Canada, Lakes Research Branch, National Water Research Institute, Burlington, Ontario.

Snowpack has been sampled at a number of sites throughout the Canadian Arctic over the past several years and the annual deposition rates of a variety of trace organics have been calculated. During the winter of 1990-91, large volume snow collectors were used to investigate deposition more intensively. These results are compared.

The fate of many of the semi-volatile compounds upon ripening and melt of the snow pack is a more complex problem. Although previous work has shown that the less water soluble, non particle associated compounds are released in first melt, it is evident in the Arctic that large quantities (perhaps as much as 90% by concentration) of many of these compounds are revolatilized. Some of the factors controlling this exchange and the significance of these findings are compared.

MONITORING OF CONTAMINANT LEVELS IN THE ENVIRONMENTAL COMPONENTS OF THE SIBERIAN SHELF SEAS. RESULTS OF FIVE YEAR STUDIES.

S. Melinkov, The Arctic and Antarctic Research Institute, Leningrad, USSR.

Levels of organochlorine pesticides (hexachlorocyclohexane, DDT) have been measured in snow, ice and water from coastal Arctic regions in the Soviet Union and from ice islands in the central Arctic Ocean. This presentation will compare the annual variability of the concentrations of the major pollutant groups and other relative information.

PATTERNS AND TRENDS OF CONTAMINANTS IN THE ECOSYSTEMS OF THE CHUKCHI AND BERING SEAS. S.M. Cheryak, Institute of Global Climate and Ecology, Moscow, USSR.

The accumulation of chlorinated hydrocarbons (CHCs) in seawater, plankton, neuston, benthic organisms and fish was investigated in the Bering Sea in 1988 during the 3rd Soviet-American ecological expedition on board the vessel "Akademik Korolev." The DDT family was the only group of CHCs whose concentration had declined in seawater in comparison to similar studies in 1984. Concentrations of DDT remained high in suspended matter with accumulation factors as high as 1000 in some surface collections. The general PCB concentration had not changed over five years. Di and trichlorobiphenyls were the most abundant in water samples and more highly substituted congeners predominated on particles. The chlordanes were identified in the Arctic region for the first time at concentrations in many samples exceeding those of the DDT group. A study of the vertical distribution found all CHCs reached depths of several thousand meters. On the basis of the data it was calculated that the Bering Sea water contained 1.6 t PCBs, 32.5 t of HCH isomers, 42 kg of DDTs and 82 kg of chlordanes. Bottom sediments accounted for 1.8 t DDTs and 6 kg of HCH. The PCB mass discharged into the ecosystem is distributed in equal portions between the waters and the upper layer of the bottom sediments.

BIOGEOCHEMICAL STUDIES OF PCB IN ARCTIC ECOSYSTEMS. A.V. Tysban, S.M. Cheryak and G.V. Panov, Institute of Global Climate and Ecology, Moscow, USSR.

The microbial and photochemical transformation of PCBs in natural marine conditions was studied during the Soviet-American ecological expedition in 1988 in the eastern, northern and southern parts of the Bering Sea and in the southern Chukchi Sea. Preliminary studies of microbial decomposition of Aroclor 1232 showed that 19 out of 70 congeners were transformed in sea water and these became the focus of the study. In the "east" area consumption of individual congeners varied from 7% for hexachlorobiphenyl to 95-100% for dichlorobiphenyls during 21 d. After the first day bacterial numbers did not increase suggesting adaptation of the bacterial community. During the next 10 d bacterial numbers increased exponentially reaching 1.8×10^4 cells/mL. After 10 d the proportion of PCB congeners remained unchanged. Destruction of the congeners was dependent on steric configuration and Cl substitution. Photochemical studies showed that only 16 Aroclor 1232 components were significntly altered in seawater. After 6 h of exposure up to 23% of 2,2',3,4-tetrachlorobiphenyl and 10-15% of certain tri- and tetrachlorobiphenyls were degraded. Many of the PCB congeners undergoing photochemical degradation were not the ones undergoing decay from exposure to microflora.

ACCUMULATION OF CHLORINATED HYDROCARBONS AND HEAVY METALS IN HIGHER TROPHIC LEVEL ORGANISMS OF THE BARENTS SEA ECOSYSTEM. T.N. Savinova, Marine Biological Institute, Murmansk, USSR.

Data on the chlorinated hydrocarbon content in organs and tissues of various sepcies of seal gulls (Larus argentatus, L. marinus, Tissa tridactyla) collected in 1979 and 1989 on the shore of the eastern Barents Sea, as well as data on the level of contamination in their food items will be presented. Polychlorinated biphenyls, organochlorine pesticides as well as DDT and metabolites were determined in all samples by gas chromatography with electron capture detection following confirmation and identification of the compounds by GC-MS.

Heavy metals content in organs and tissues of the same species of seal gulls, fulmar (Fulmarais glacialis), Havellc (Clangula hycmalis) and young harp seals (Phagophilus groenlandica) was studied by AAS method. The concentrations of the heavy metals that were detected reflected normal physiological-biochemical processes and did not suggest high levels of contamination.

Differences in the levels of bioaccumulating contaminants connected with the ecology of the species will be discussed.

ORGANOCHLORINE LEVELS IN ARCTIC RINGED SEALS, 1972-1989. R.F. Addison, Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Onva Scotia.

Ringed seals (Phoca hispida) have been sampled from the same population at Holman Is., NWT, in 1972, 1981 and 1989. Blubber concentrations of polychlorinated biphenyls (PCBs) have declined to about 20% of their initial value between 1972 and 1989, presumably reflecting the bans on manufacture and use of these materials imposed during th 1970's. DDT-group residues have declined less dramatically suggesting that there may be a continuing supply of DDT to the western Arctic. More detailed analyses of trends in other residues and in chlorobiphenyl congeners will be discussed.

CIRCUMPOLAR SURVEY OF ORGANOCHLORINES IN POLAR BEARS: PRELIMINARY RESULTS.

R.J. Norstrom, Environment Canada, Canadian Wildlife Service, Hull, Québec.

A survey of contaminants in fat of the polar bear (*Ursus maritimus*) throughout its circumpolar range was initiated in 1989. Approximately 700 samples were collected from Canada, Alaska, Greenland, Svalbard and the USSR. Many of the samples were 3 mm skin biopsy punches from tranquilized bears. Approximately half of the samples have been analysed for 37 organochlorines, including 12 chlordane and 16 PCB congeners. Preliminary analysis of the available data, uncorrected for samples size, condition, age and sex, indicated that chlordane was evenly distributed geographically, whereas PCB levels were significantly higher in Svalbard than in most other areas. Levels of these residues ranged from 2 to 20 mg/kg lipid. PCB congener patterns indicated an increasing relative contribution from Aroclor 1254 sources from east to west in Canada. DDE exhibited a strong tendency to increase in the same direction (0.05 to 0.45 mg/kg), probably indicating a North American source. HCBz was distributed similar to chlordane, but highest levels of TeCBz and PnCBz (0.02 to 0.2 mg/kg) were found in the Canadian high arctic archipelago, similar to that of TCDD, possibly indicating transpolar sources from Eurasia. Only a-HCH exhibitetd a clear tendency to decrease from west to east (0.45 to 0.1 mg/kg), indicating an Asian source.

THE U.S. ARCTIC CONTAMINANT RESEARCH PROGRAM: CHEMICAL SEDIMENT STRATIGRAPHIES OF TWO ALASKAN LAKES.

C.P. Gubala, D.H. Landers, M. Monetti and R. Thomas, Mantech Environmental Services, Corvallis, Oregon, and U.S. EPA, Environmental Research Laboratory, Corvallis, Oregon, and U.S. DOE, New York, New York, and U.S .EPA, Cincinnati, Ohio.

A research segment of the U.S. Arctic Contaminant Research Program is designed to yield estimated trends in historical pollutant fluxes through the examination of lake sediment stratigraphies. This record is essential to reveal the recent and baseline accumulation rates of toxic constituents that may then be correlated with those from other environmental compartments to refine the integrated compartments to refine the integrated perspective on U.S. Arctic contamination.

In April of 1991, sediment cores were collected from two Alaskan lakes; Wonder Lake (63°28'N, 150°52'W; Denali National Park and Preserve) and Lake Schrader (69°22'N, 144°60'W; Arctic National Wildlife Refuge). Depositional chronologies of chlorinated hydrocarbons and trace metals will be presented and discussed for the two systems.

ACCUMULATION OF COPLANAR PCBs IN ARCTIC MARINE MAMMALS AND FISH.

D.C.G. Muir, C.A. Ford, M.D. Segstro, R.J. Norstrom and M. Simon, Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba, and Environment Canada, Canadian Wildlife Service, Hull, Québec.

Concentrations of toxic non-ortho and mono-ortho substituted (coplanar) PCBs were determined in marine mammals from Canada, Greenland and Alaska, and in Arctic char from the Canadian Arctic in order to assess (1) the dietary exposure of native people and (2) the possible effects on marine biota. Levels of 3,3',4,4',5-pentachlorobiphenyl (PCB-126) ranged from 41 to 607 ng/kg in beluga, 23 to 195 ng/kg in ringed seal and from 22 to 217 ng/kg in narwhal blubber. Arctic char had lower concentrations of PCB-77 and PCB 126 than marine mammals ranging from 27 to 119 ng/kg for PCB-77 (3,3',4,4'-tetrachlorobiphenyl (TCB) and PCB-126 from 5 to 25 ng/kg in whole fish. PCBs-126, 105 (2,3,3',4,4'-TCB) and 118 (2,3',4,4',5-pentachloro-) contributed greater than 98% of TCDD toxic equivalent factors (TEQ) in beluga and narwhal blubber and 70% in ringed seal blubber samples. The remaining 2 to 22% of TEQ was due to the contribution of 2,3,7,8-TCDD. Arctic char samples contained a higher proportion of non-ortho congeners (0.2% of $\sum$PCB) than narwhal or beluga (0.001-0.02%).

LEVELS OF PCDD/PCDF AND COPLANAR PCB IN BIOTA AND SEDIMENTS FROM THE NORWEGIAN ARCTIC.

M. Oeheme, M. Schlabach and A. Biseth, Norwegian Institute for Air Research, Lillestrøm, Norway.

Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) have been detected in seal blubber from the Canadian and Norwegian Arctic. However, there is still a lack of knowledge about the levels of PCDD/PCDF as well as coplanar PCB in other organisms from the Arctic.

Crabs and mussels bioaccumulate all PCDD/PCDF congners without changers in the isomer patterns. In this way, information can be obtained about the source type (e.g. combustion) and possible orgin of the found levels. Surprisingly high levels of 5-20 pg/kg 2,3,7,8-TEQ (International model) are found in crabs along the whole Norwegian coast. Concentrations in mussels are one order of magnitude lower.

Crabs and mussels were also collected from the Norwegian Arctic (ca. 66-80°N latitude) to obtain more information about concentrations and congener patterns. Furthermore, sediment samples from Spitzbergen were analyzed to compare the isomer distributions with those found in crabs. In addition, 3 polar bear milk samples were quantified. Measurable amounts of PCDD/PCDF and coplanar PCB were also found in all samples. They will be compared with results from the Norwewgian main land.

POSTERS/L'AFFICHE SCIENTIFIQUE

TOXIC EQUIVALENT FACTORS (TEFS) AND TISSUE DISTRIBUTION FOR SEVERAL 2,3,7,8-SUBSTITUTED DIOXINS IN RAINBOW TROUT.

J.L. Parrott, P.V. Hodson, D.G. Dixon and M.R. Servos, Department of Biology, University of Waterloo, Waterloo, Ontario, and Department of Fisheries and Oceans, Institut Maurice-Lamontagne, Mont-Joli, Quebéc, and Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Dept. of Fisheries and Oceans, Burlington, Ontario.

Dioxins are a group of 75 chemicals whose properties and toxicity vary greatly. Concern about environmental contamination by dioxins has developed largely because of the extreme toxicity of one congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). However, other dioxin congeners and related compounds (polychlorinated dibenzofurans and biphenyls) may be present in environmental samples in mixtures of unknown toxicity. Hence, a method is needed to estimate the toxicity of these mixtures, and the relative contribution of each component to the total toxicity.

One widely-adopted approach is the application of Toxic Equivalent Factors (TEFs; Kannan *et al.*, 1988). The TEF for each compound is estimated from its toxic potency relative to that of TCDD, the most toxic congener. For example, the toxicity of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) is 10 times less than that of TCDD. Therefore, the TEF for TCDD is 1.0 while that for HxCDD is 0.1; food containing HxCDD would present the same risk as TCDD only if HxCDD concentrations were 10 times higher.

TEFs were originally developed to estimate the risks to human health from consuming dioxin congeners. Toxicities were measured using mammalian models, usually rats, and the toxic effects measured were thymic involution and growth impairment (Poland and Knutson, 1982). Since tests of these effects are lengthy and expensive, tests of the induction of activity of liver mixed function oxygenase (MFO) enzymes were substituted. A very strong correlation exists between MFO induction and other toxic effects (Mason *et al.*, 1986). Induction can be measured quickly, both in living rats and in mammalian cell cultures, and relative potencies are equivalent to those measured by chronic tests.

While TEFs enable risk estimates for the protection of human health, can they estimate risks to aquatic organisms?

Dioxins are common contaminants of fish, but there is little information on tissue residues associated with chronic toxicity, and no information on relative potencies of different congeners. Therefore, to test whether mammalian TEFs can estimate dioxin risks to fish, we measured the relative potencies of five dioxin congeners for liver MFO induction in rainbow trout (Onchorynchus *mykiss*).

Groups of 200 g rainbow trout were given single oral doses of dioxin in gelatin capsules. After 2, 4, 8 or 16 days, individuals were subsampled from exposed and control (carrier only) groups and their liver ethoxyresorufin-O-deethylase (EROD) activity was measured fluorimetrically (Pohl and Fouts, 1980). The dioxins tested were: 2,3,7,8-^{3}H tetrachlorodibenzo-*p*-dioxin (TCDD; 0.06-2 µg/kg), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PnCDD; 0.03-10 µg/kg), 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,6,7,8-HxCDD; 0.3-8 µg/kg), 1,2,3,4,7,8-^{14}C hexachlorodibenzo-*p*-dioxin (HxCDD; 0.1-10 µg/kg) and 1,2,3,4,6,7,8-^{14}C heptachlorodibenzo-*p*-dioxin (HpCDD; 0.3-80 µg/kg).

Tissue burdens of TCDD, HxCDD and HpCDD, compounds labelled with tritium or ^{14}C, were measured by scintillation counting of muscle, whole liver, blood serum, liver fat and the liver enzyme extract (post-mitochondrial supernatant or PMS). The PMS contained 25-50% of TCDD, 10-60% of the HxCDD and 30-60% of the HpCDD found in the whole liver, but the liver contained <2%, <6%, or <5% respectively of the administered doses of the three compounds. For TCDD and HxCDD, the PMS and whole liver concentrations increased with dose administered, but this was not the case for HpCDD. Skeletal muscle, blood serum and liver fat contained up to 20%, 3% and 0.05% of the administered dose of TCDD respectively.

Dioxin exposure caused liver EROD activity to increase up to 300 fold in a dose-dependant fashion. TCDD at all doses caused very rapid induction (within 2 d). High doses of HxCDD and HpCDD caused induction within 2 d, but lower doses required 4 d to fully induce. Enzyme activity remained elevated throughout the 16 d and maximal activity was about 600 pmol mg protein^{-1} min^{-1} for all compounds.

Control EROD activity (2.0 ± 1.9 pmol mg protein^{-1} min^{-1}; mean ± s.d.) did not vary seasonally (May 1990 to Feb 1991) and there was no enzyme induction by the solvent used in the gelatin capsule. Over the 16 d sampling period, there was insufficient excretion of dioxin by dosed fish to induce control fish held in the same tank; EROD activity of these control fish was the same as that of unexposed controls held in a separate tank.

TEFs were calculated by comparing the minimum effective dose (EDM) for each dioxin to the EDM for TCDD. EDMs were the minimum doses causing statistically significant increases in EROD activity above control values. PnCDD was almost as potent an inducer as TCDD (Table 1), but both HxCDDs were less potent with TEFs of 0.2; the TEF of HpCDD was 0.04. TEFs decreased with increasing molecular size and degree of chlorine substitution as previously observed with mammals (Bradlaw *et al.*, 1980). TEFs did not change greatly when EDMs were expressed as the dose measured in the PMS rather than as the whole-body dose administered by gelatin capsule.

Table 1. Comparison of dioxin TEFs for rainbow trout (calculated using oral dose or PMS concentration) with international TEFs based largely on mammalian data.

	TCDD	PnCDD	1,2,3,6,7,8-HxCDD	1,2,3,4,7,8-HxCDD	HpCDD
DOSE GIVEN[1] µg/kg	0.1	0.12	0.55	0.62	2.3
TEF	1	0.83	0.18	0.16	0.043
PMS CONCN.[2] pg/g	10			53	430
TEF	1			0.20	0.023
International TEF[3]	1	0.5	0.1	0.1	0.01

1. Estimated oral doses necessary to raise EROD activity significantly above controls.
2. Estimated amount of dioxin in post-mitochondrial supernatant (PMS) from 1 g liver necessary to raise EROD activity significantly above controls.
3. From NATO/CCMS, 1988.

All dioxins tested were more potent than predicted by conventional TEFs developed using mammalian species (international TEFs, Table 1). The fish TEFs were 66-330% higher than the corresponding mammalian TEFs, suggesting that risks of dioxins to fish would be underestimated if judged from mammalian TEFs. These data suggest that fish TEFs would be most suitable for judging risks to fish and that more congeners should be tested for EROD induction. However, before fish TEFs can be fully accepted, the correlation of potency for fish liver MFO induction and potency for chronic toxicity must be established.

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ACID VOLATILE SULFIDE (AVS) IN A SEASONALLY ANOXIC MESOTROPHIC LAKE: SEASONAL AND SPATIAL CHANGES IN SEDIMENT AVS.

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ABSTRACT

Acid Volatile Sulfide (AVS) is an operational definition for the sulfides removed from sediment by cold acid extraction, and consists mainly of H_2S and FeS. It has been proposed that this fraction could be used as a sediment parameter for divalent metal toxicity (eg. Cd, Ni, Hg), due to its ability to bind metals and hence render them unavailable to biota. However before it can be used as a predictive tool, we must develop a better understanding of its biogeochemical nature especially in fresh water systems, where relatively few studies have been conducted. An in depth study is presented of AVS concentrations in a seasonally anoxic mesotrophic lake (Williams Bay, Jack Lake, Ontario), with seasonal, spatial and sediment AVS profiles. Sediment handling, storage and modifications to AVS extraction techniques used are discussed, and comparisons made to AVS in other lakes, with reference to its utilization as a sediment toxicity parameter.

INTRODUCTION

Acid Volatile Sulfide (AVS), is an operationally defined sediment parameter that consists of sediment sulfides that are cold acid soluble, mainly free sulfides and amorphous iron monosulfides (Herlihy, et.al. 1985; Thode-Andersen and Jorgensen 1989). It has been utilized for many years in the determination of sulfur reduction and diagenesis products (eg. Oenema 1990; Smith and Klug 1981; Nriagu 1968), and along with Chromium Reducible Sulfur (CRS), makes up the pool of total reduced inorganic sulfur (Fossing and Jorgensen 1989).

Although acid volatile sulfides have been studied for many years, it is only recently that they have been recognized as a possible indicator of metal toxicity in sediments, due to sediment partitioning of metals (Di Toro et.al. 1991). The chemical basis for this lies in the fact that sulfides have a very high affinity for divalent metals, and metals in metal sulfides that have a lower solubility product than FeS ($4*10^{-19}$), can often displace the iron from its sulfide (Phillips and Kraus 1965). The new metal sulfide formed in this displacement reaction (eg.CdS, NiS), will precipitate out of solution, limiting its bioavailability (Gardner and Gunn 1989).

Di Toro et. al.(1991), conducted sediment toxicity tests with cadmium and nickel on marine and freshwater organisms using sediments with different AVS concentrations, and concluded that "no significant mortality occurs relative to controls if the acid volatile sulfide concentration is greater on a molar basis than the simultaneously extracted metals".

Because of the high sulfate concentrations of seawater, the majority of AVS work has been done on marine sediments, but the few AVS measurements known for freshwater sediments (eg. Di Toro et.al. 1990; Nraigu 1968), indicate that significant AVS concentrations may exist there also. The problem, as always with lake systems, is when and where in the lake should the AVS be measured, and how can it be applied to a whole lake

system?

The production of AVS is bacterial mediated (Oenema 1990), and depends on sulfur/sulfate concentrations, a metabolizable carbon source and redox conditions. These factors plus others are liable to make the measurement of AVS both seasonally and spatially variable in most dimictic freshwater lakes.

It was the object of this study to elucidate these problems, with a preliminary investigation of AVS in freshwater sediments. Temporal, spatial and between lake variability was examined, to determine if AVS measurements could be used predictably on a whole lake basis.

Methods

Study Sites

Three study sites with different morphological and biological features (Table 1), were selected to study spatial variation in AVS. All three lakes had seasonally anoxic hypolimnions, but with varying periods of stratification and productivity.

Crosson a small lake 140 km north of Peterborough, Ontario, and the least productive of the three lakes, was sampled at 1, 8, 10.5, 15 and 20 m August 8th 1991. Gullfeather, similar in most respects to Crosson (eg. size, location, pH, DOC), was sampled August 14th, 1991 at 4, 6, 8, 10, and 12 m.

The third lake, Jack Lake (Williams Bay), is a mesotrophic lake, situated on precambrian bedrock of the Canadian shield. It was the most productive of the three lakes, and was used for seasonal as well as spatial studies, as it was hypothesized that if seasonal differences did occur that they would be greatest in the most productive lake. Cores were taken from Jack Lake approximately biweekly from April 23rd to August 12th, 1991 (112 day period), for the seasonal study, and transect cores at 18, 20 and 22 m were taken June 4th, 1991, for the spatial comparison.

All cores were taken by KB-Corer, using acrylic core tubes, inside diameter 10 cm. Oxygen and temperature profiles for Jack Lake were recorded during the sampling period, and morphometric data was taken from Carignan and Lean (1991). Oxygen profiles and morphological data for Crosson and Gullfeather were obtained from OMOE Data Reports (Nicolls et.al. 1983; Reid et.al. 1983).

Handling of Sediments

All cores were transported back to the lab, where the top 15 cm were extruded under nitrogen, homogenized and analyzed for AVS within 24 hours. It was found that sediment extruded in an oxidizing atmosphere, or left for long periods of time, was consistently lower in AVS, due to degassing and oxidation of reduced iron monosulfides.

AVS Analysis

Acid Volatile Sulfides are, as the name implies, the sulfides released when sediments are exposed to acid. As yet no standard method has been developed(eg. Di Toro et.al. 1990; Cutter and Oatts 1987; Davison and Lishman 1983), but it is likely, due to the simplicity of the component being measured, that most methods are comparable in accuracy, if not in detection limits and precision. According to Di Toro et.al. (1990), the lower limits of the

Table I.Morphometric and biological characteristics of the three study sites. OMOE Data Report Series. *Carignan and Lean, 1991. **This study.

	Jack Lake (Williams Bay)	Crosson	Gullfeather
Lat	4442	4505	4506
Long	7802	7902	7901
Area (ha)	1236.7 (80)*	56.7	65.9
Mean depth (m)	8.8*	8.4	4.8
Zmax (m)	21.7*	25	13
ph	7.8	5.8	6.0
DOC(mg/l)	5.0	4.2	4.2
Fe (mg/l)	-	270	350
LOI(%)**	47	41	38
Wet:Dry wt.of sediments**	36.2	13.1	14.5

applicability of AVS as a metal toxicity parameter is about 1 umol/gram of sediment, due to other partitioning phases gaining importance in more oxidized sediments. Therefore as long as detection limits and experimental variation fall well below this, most methodologies should give acceptable results. The main thing is to avoid oxidation of the sediments, and to keep the concentration of the acid used to release the sulfides low. Most methods use 0.5 to 1.0 M HCl, as a higher concentration may release sulfides from other species such as greigite (Cutter and Oatts 1987).

To avoid oxidation of sediments all handling and core extrusions were carried out in an anaerobic atmosphere, using nitrogen gas passed through an oxygen scrubbing system (Di Toro et.al. 1990), to remove any residual oxygen.

Three 250 ml flasks were connected in series by air-tight stoppers and the appropriate glass and tygon tubing. With the reaction flask (flask 1) left empty, and 75 ml of Sulfide Anti-Oxidant Buffer (SAOB, Orion), placed in flasks 2 and 3, the system was initially purged with nitrogen for 30 minutes. Homogenized sediment (15 ml), was added by syringe to the reaction flask, followed by 100 ml 1M HCl. Nitrogen continued to purge the system for 45 minutes, during which time the released sulfide was trapped by the SAOB in flask#2. No sulfide was detected in flask#3. The S^{2-}concentration was measured using a silver-sulfide electrode (Orion model #94-16), and calculated using the appropriate standards, titrations and calibration curves (Orion manual, Ag^{+}/S^{2-} electrode).

This method for measuring AVS has not, to the authors' knowledge, been documented elsewhere, and is well worth mentioning due to its speed and comparative simplicity. It has a detection limit of 0.1 ppm, below which electrode readings fluctuate too greatly for accurate readings to be made. All blank runs fell below this value. The yield was 95%

(standard deviation 10%), using known FeS standards. For the AVS/sediment depth profile (Fig.3), one 15 ml samples was analyzed at 2, 3, 4, 6, 8, 10, 15, 20, 25 and 40 cm, plus the sediment/water interface. All other values are the mean of three replicates, except for April 23rd (Fig.2), which was the mean of two replicates

All results are quoted as umol S^{2-} evolved per gram of wet sediment (w.s.), the average wet weight:dry weight ratio is shown in Table 1. Separate samples were taken for wet:dry weight ratios and LOI.

Results

Spatial AVS Patterns

The comparison between the three lakes (Fig.1), revealed variation both between and within lakes, indicating a positive relationship between both productivity and AVS, and depth and AVS. The least productive of the lakes, Crosson, had very little AVS at any depth. Variation in AVS between Gullfeather and Crosson, may be due to differences in their productivity, or their oxygen profiles. Oxygen profiles for Gullfeather (Reid et.al. 1983), indicate that it is well stratified by mid-August, with very little oxygen in the water below 6 m, while profiles for Crosson show a different pattern with anoxia occurring late in the season (Sept-Oct), and only below 17 m. Maximum AVS values for Crosson and Gullfeather both occurred at the deepest point sampled, and were 0.142 and 0.884 umol S^{2-}/g w.s. respectively. Jack Lake had a maximum value of 2.7 umol S^{2-}/g w.s., again at the deepest point sampled.

It should be noted that oxygen was still present at 18 m (4.6 mg/l), but not at 21 m on June 4th when this transect was taken.

The sediment depth/AVS profile (Fig.3), for Jack Lake August 12th, indicated that most of the AVS occurred in the first 15 cm, with a peak at about 8 cm, this validates the decision to use the top 15 cm in our analysis. Similar AVS profiles are reported elsewhere (Davison and Lishman 1983; Herlihy and Mills 1985).

It was noted that there was also a difference in porosity between Jack Lake and the other two lakes based on the wet:dry ratios. The additional pore water may be instrumental in trapping sulfides, which can then precipitate with reduced iron, and so increase the AVS concentration. Carignan and Lean (1991), observed that sulfide concentrations in Jack Lake sediment profiles appeared to be controlled by the formation of iron monosulfides, with low H_2S in the deepest sediments due to an excess of Fe^{2+}. Fe and Mn enrichment of profundal sediment porewaters has been reported previously (Tessenow 1975).

Seasonal AVS Patterns

AVS analysis on Jack Lake sediments, and oxygen readings from spring turnover until mid-summer (April 23rd to August 12th, 1991), indicated that there was a significant increase in AVS as conditions became more reducing (Fig.2), due to oxygen depletion in the hypolimnion. Just after turnover the AVS was still very high (>4 umol S^{2-}/g w.s.), this quickly changed to reach a low of 1.24 umol S^{2-}/g w.s. (May 13th), before summer stratification and anoxia. By August 12th the AVS was again over 4 umol/g, and the lake was totally depleted of oxygen below 11 m. Bell et.al.(1990), reported a similar rapid increase in AVS during the establishment of anaerobic reducing conditions in lake sediment after a storm had introduced oxidized sediment.

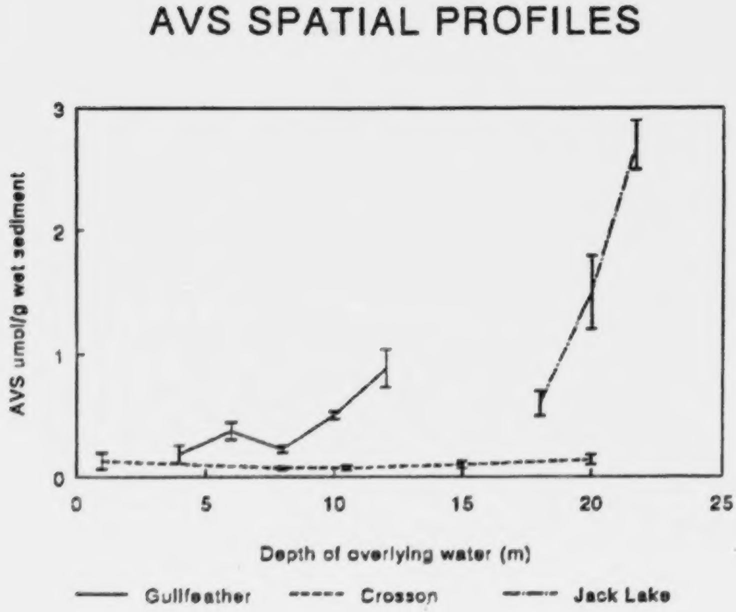

Figure 1. AVS transect profiles of the three study sites. The error bar represents 2 standard deviations. n=3.

Discussion

It is evident from this study, that the possibility of using AVS as an indicator of sediment metal bioavailability in freshwater systems, will only be recognized when whole lake estimates can be made predictably.

AVS concentrations on a whole lake basis are primarily controlled by, 1)the productivity of the lake, 2)the availability of a reducible source of iron and sulfate, and 3)the morphometry of the lake. To make accurate predictions regarding whole lake AVS, more data will have to be collected, so that the relationship between AVS and these parameters can be modelled.

Because AVS changes with depth in the sediment, sampling site in the lake and between lakes in the same geographic area, we are left with the question of where and when to sample it? The answer to this will no doubt include position in the lake and time spent anoxic. If maximum values are desired, they can be obtained by sampling at the end of the longest period of stratification, and in the main depositional basin of the lake. This however may, or may not be representative of the lake as a whole.

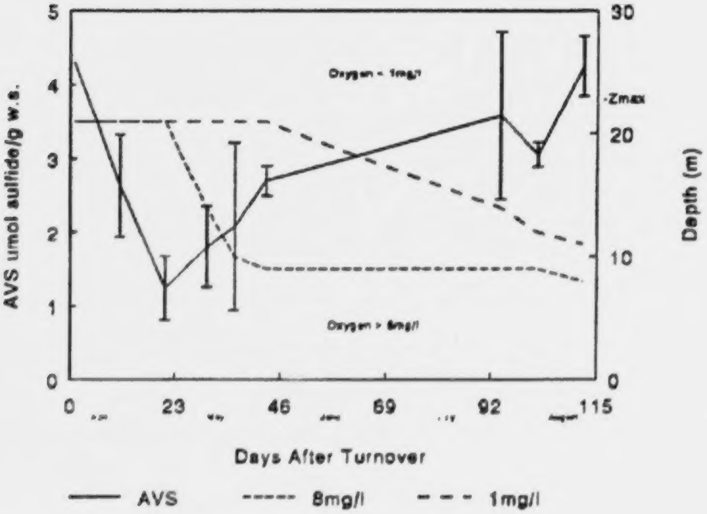

Figure 2. Seasonal AVS concentrations, and partial oxygen profile at 21 m, in Jack Lake from April 23rd to August 12, 1991. Error bars represent 2 standard deviations. n=3.

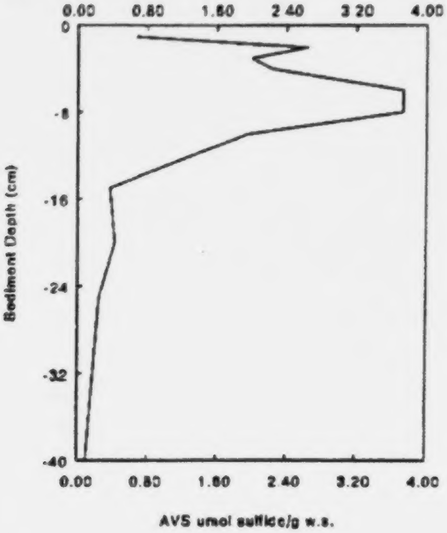

Figure 3. AVS concentration with sediment depth in Jack Lake August 12th 1991, at 21 m.

The relevance of seasonal oxic conditions will have to be taken into consideration if AVS is to be used as an indicator of metal toxicity in freshwater lakes, as during mixis metal sulfides can become oxidized. The extent to which this occurs will depend on the solubility product of the sulfide and on the period of time between the turnover event and the return of anaerobic reducing conditions.

Based on the present study, AVS should be high enough to limit the bioavailability of divalent metals in mesotrophic lakes, where the hypolimnion is anoxic for most of the year.

Continued studies on Jack Lake are aimed at predicting AVS concentrations with oxygen depletion models, and extending it to the whole lake system.

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RÉPONSES BIOCHIMIQUES AU CADMIUM CHEZ *Anodonta grandis* EN MILIEU AQUATIQUE.

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RÉSUMÉ

L'évaluation de l'impact des contaminants sur la macrofaune est sujette à la connaissance des populations qui vivent dans un écosystème perturbé. Dans une expérience de transfert d'*Anodonta grandis*, nous avons réalisé en 1990 un suivi saisonnier de myes d'un lac témoin (COP), de myes transférées dans un lac pollué au Cd (TVA) et de myes indigènes de ce lac (CVA). Pour une même classe d'âge, de juin à octobre, les effets chroniques du Cd se manifestent, en CVA, par des poids de chair, de gonades et de manteau de 30% plus faibles qu'en COP et TVA et des teneurs en protéines, lipides et glycogène dans les gonades et en lipides et glycogène dans la glande digestive, inférieures à celles mesurées chez les myes de TVA et COP. Les effets aigus du Cd chez le groupe TVA s'observent par une perte de poids du manteau et d'une baisse du rapport ARN:ADN. Cette expérience de transfert a donc permis de préciser certains effets délétères du Cd *in situ* et de valider l'utilisation de sondes bioanalytiques dans une approche écotoxicologique.

INTRODUCTION

Plusieurs effets délétères de contaminants chez les mammifères ont été reliés ces dernières années à leur induction des processus de peroxydation des lipides, à la perturbation des lipides membranaires par les polluants lipophiliques (Bach et Sela, 1984; Goel *et al*, 1988) et à la production finale de malonyl dialdéhyde (MDA) (Svingen, 1979). Le mercure (Stacey et Kappus, 1982), le nickel (Sunderman *et al*, 1985) et l'étain (Dwivedi *et al*, 1984) exerceraient leur toxicité par la peroxydation des lipides en relation avec les dommages cellulaires qui en résulteraient (Kinter et Pritchard, 1977) alors que la glutathione (GSH), les sucres et la vitamine E protégeraient les cellules hépatiques du rat des effets toxiques du mercure (Welsh, 1979).

Toutefois, les informations sont rares en ce qui concerne les mécanismes d'induction de la peroxydation des lipides chez les invertébrés. Des études récentes chez *Mytilus galloprovincialis* montrent la présence de la malonyl dialdéhyde (MDA) dans la glande digestive, les branchies et le manteau et des concentrations importantes de GSH dans le manteau (Ribera *et al*, 1989).

Les effets toxiques des contaminants et principalement des métaux lourds, s'expriment donc en tout premier lieu au niveau biochimique (Blackstock, 1984) et les perturbations métaboliques qui en résultent peuvent modifier les indices de condition et les réserves énergétiques (Pelletier et Pellerin-Massicotte, 1990). Les informations recueillies par ces mesures peuvent être complétées par la mesure des acides nucléiques et le calcul du rapport ARN:ADN, un indicateur rendant compte de l'effort de l'organisme vers la production somatique (Wright et Hetzel, 1985).

Les objectifs de cette recherche sont donc de vérifier la présence des mécanismes d'induction de la peroxydation des lipides chez une moule d'eau douce *Anodonta grandis*, présente dans un milieu lacustre fortement contaminé au cadmium et de relier les teneurs en malonyl dialdéhyde avec la condition physiologique de ces myes d'eau douce.

MATÉRIEL ET MÉTHODES

Les critères qui ont mené au choix d'Anodonta grandis pour cette étude furent les suivants: elle est abondante dans les milieux lacustres de la région de Rouyn-Noranda, de grande taille et possède comme tous les bivalves, une bonne capacité de bioaccumulation des métaux. Les myes ont été échantillonnées dans le lac Opasatica qui comporte des teneurs moindres en cadmium que le lac Vaudray. Des myes entre 3 et 5 ans, et 20 à 25 par jour d'échantillonnage ont été placées dans des enclos dans les lacs Opasatica (COP) et Vaudray (TVA). Des myes témoins du lac Vaudray ont été placées également dans des enclos dans leur lac d'origine. Au jours 0, 30, 60 et 90 après le transfert, les organismes étaient échantillonnés, pesés et mesurés. Les myes étaient alors expédiées par avion au laboratoire, dans des sacs de polyéthylène remplis d'eau à 4° C. A leur arrivée, les myes étaient disséquées et les poids totaux de chair, manteau, gonades et branchies étaient notés et les tissus étaient par la suite homogénéisés immédiatement dans les tampons adéquats.

Les mesures de l'ARN et de l'ADN ont été réalisées dans les branchies selon la méthode de Wright et Hetzel (1985). Les sucres totaux, protéines, glycogène et lipides ont été mesurés dans les manteau, les gonades et la glande digestive selon les méthodes de Dubois (1956), Bradford (1976), Carr et al. (1984) et Frings et al. (1972).

Les données des mesures pondérales et biochimiques étaient analysées en premier lieu pour vérifier la normalité de la distribution par un test de Kolmogorov-Smirnov et l'équivariance était par la suite vérifiée par le test de Bartlett en utilisant le logiciel SYSTAT pour IBM-Pc. Des test paramétriques ou non-paramétriques étaient alors appliqués.

RÉSULTATS

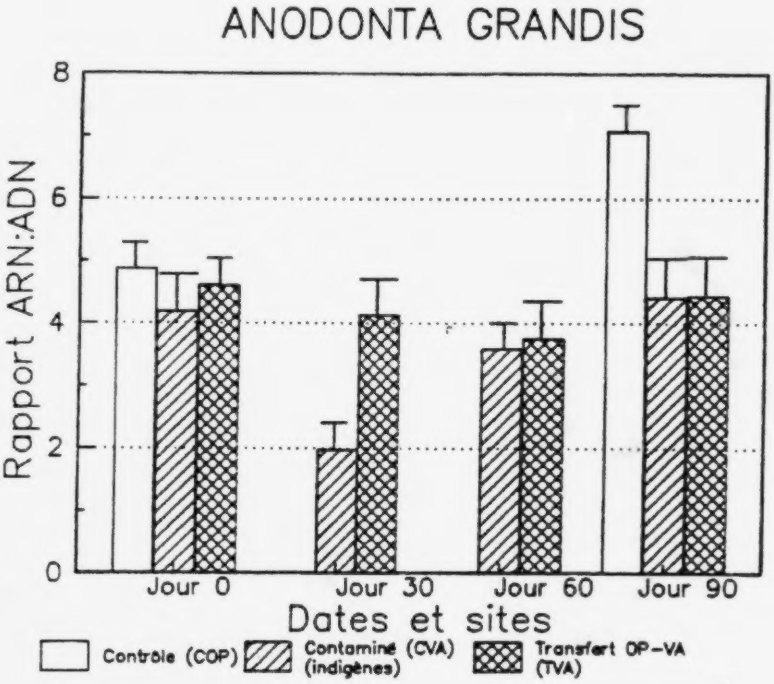
Pour des âges similaires, les myes indigènes du site contaminé (CVA) ont le poids total, le poids de chair, des branchies et le poids des gonades significativement inférieurs ($p < 0.05$) à ceux du site contrôle (COP) et des myes transférées (TVA). Les myes transférées (TVA) ont pour leur part montré une diminution significative de leur poids total, du poids de chair et de gonades au jour 60 de l'échantillonnage, pour revenir au niveau des myes contrôles du site Opasatica au jour 90. Par contre, les rapports poids de gonades / poids de chair ne varient pas selon les sites ou les dates d'échantillonnages. Le poids de la glande digestive a tendance à augmenter graduellement chez les myes (COP et TVA) entre les jours 0 et 90.

Les sucres totaux dans la glande digestive et les gonades diminuent entre les jours 0 et 90 chez les myes du site contrôle mais demeurent élevés dans le site contaminé (CVA et TVA). Les lipides de la glande digestive sont plus abondants dans le groupe de myes transférées du site Opasatica vers le lac Vaudray que chez les myes indigènes de ce dernier lac. Par contre, les lipides gonadiques ne varient pas significativement d'un site ou d'une date d'échantillonnage à l'autre. Les réserves hépatiques en glycogène baissent proportionnellement durant la saison estivale chez les groupes COP et TVA alors que le groupe CVA montre des teneurs significativement inférieures au début de la saison, pour atteindre des niveaux semblables à ceux des autres groupes au jour 90. Le glycogène dans les gonades ne varie pas significativement dans les trois groupes étudiés.

La malonyl dialdéhyde (MDA) ne montre pas de variations significatives entre sites et dates d'échantillonnage dans la glande digestive, mais les teneurs sont légèrement inférieures dans le groupe CVA pour tous les jours de prélèvement.

Le rapport ARN:ADN dans le manteau d'Anodonta grandis (Figure 1), est similaire pour tous les groupes au jour 0, mais montre une diminution importante aux jours suivants chez le groupe CVA. Le groupe de myes transférées vers le lac contaminé ne montre pas de baisse de ce rapport avant le jour 60, et les valeurs sont similaires pour les groupes CVA et TVA au jour 90 mais grandement inférieures au groupe contrôle COP.

Figure 1. Etude des variations du rapport ARN:ADN en fonction d'un gradient de pollution au cadmium.



DISCUSSION

Ces résultats démontrent la relation directe entre la baisse de production somatique et la quantité d'ARN dans les cellules d'Anodonta grandis. En effet, pour les myes présentes dans le lac contaminé au cadmium, l'effort de production somatique normalement retrouvé pendant la saison estivale et reflété par les mesures pondérales et le rapport ARN:ADN, est entravé probablement par la présence de métaux lourds. L'indice de condition des gonades et les concentrations gonadiques en lipides et en glycogène demeurant constants pour les trois groupes de myes, les effets délétères toucheraient plutôt la croissance que les fonctions de reproduction.

Des conditions environnementales adverses peuvent toutefois affecter la production somatique mais des études nombreuses ont établi une relation directe entre les teneurs en métaux lourds et une croissance déficiente ((Pelletier et Pellerin-Massicotte, 1990). Pour des myes adaptées à un milieu lacustre peu contaminé, le transfert vers le lac Vaudray a entraîné une chute du poids de certains organes et du rapport ARN:ADN au jour 60 de l'expérience de transfert. Cette diminution des mesures pondérales et de l'effort de production somatique pourrait s'expliquer par la période de ponte qui a lieu normalement au mois d'août chez Anodonta grandis.

La présence de cadmium au lac Vaudray modifie les paramètres énergétiques des myes indigènes et des myes transférées. En effet, les sucres totaux sont en concentration importante chez ces bivalves, révélant leur importance dans les mécanismes de défense contre les radicaux libres oxygénés (Ribera et al, 1989) et aussi en périodes de stress car ils constituent une réserve énergétique facilement utilisable (Pellerin-Massicotte et al, 1989b).

Par contre, les teneurs en malonyl dialdéhyde sont comparables pour tous les groupes, ce qui tend à exclure l'hypothèse du stress oxydant. Cependant, les études in situ chez les invertébrés étant rares, nous ne connaissons pas encore la cinétique d'induction et d'apparition de la malonyl dialdéhyde. Le plan d'échantillonnage adopté n'est peut-être pas adapté à ce paramètre biochimique. Nous sommes actuellement, dans le cours de travaux complémentaires, à préciser la variabilité saisonnière et individuelle de la malonyl dialdéhyde et nous prévoyons étudier en détail ses paramètres cinétiques en milieux lacustre et marin. Les réserves lipidiques et en glycogène de l'hépatopancréas étant nettement inférieures chez le groupe indigène du lac Vaudray, ces myes présentent donc une condition physiologique déficiente.

Des études antérieures réalisées dans nos laboratoires ont démontré que les lipides et le glycogène sont les paramètres biochimiques les plus sensibles aux contaminants (Pellerin-Massicotte et al, 1989a,1989b; Pelletier et Pellerin-Massicotte, 1990) et ce travail démontre également la pertinence d'utiliser le rapport ARN:ADN pour préciser les effets délétères des contaminants sur la production somatique. Ces indicateurs biochimiques s'avèrent donc de bons indicateurs d'une pollution sous-létale.

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BIOCONCENTRATION ET DISTRIBUTION DE $^{54}Mn^{2+}$ ET EFFETS D'AGENTS COMPLEXANTS CHEZ LA TRUITE BRUNE, Salmo trutta.

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RÉSUMÉ

Nous avons étudié les effets des acides humique et fulvique, de l'éthylxanthate de potassium (PEX), du diéthyldithiophosphate de sodium (SEP) et du diméthyl- et du diéthyldithiocarbamate de sodium (SMC et SEC) sur la bioconcentration et la distribution de $^{54}Mn^{2+}$ chez Salmo trutta. Des groupes de 7 poissons ont été exposés pendant une semaine à 0,1 μg Mn·L^{-1} (1 μCi·L^{-1}) avec et sans agents complexants. Cinq poissons par groupes ont été disséqués et la radioactivité dans les divers organes et tissus a été déterminée par comptage gamma. Les deux autres poissons ont été utilisés pour la macroautoradiographie. Le coefficient de partition octanol-eau du Mn avec ou sans agents complexants a aussi été déterminé. Les poissons exposés au Mn seulement ont accumulé 0,73 ± 0,04 ng Mn·g^{-1}. Les acides humique et fulvique ont eu peu d'effets sur la bioconcentration et la distribution du Mn. Les autres agents complexants ont diminué de 40% la bioconcentration de Mn. La distribution du Mn a aussi été affectée; une plus grande proportion du Mn se retrouve dans les viscères tandis que cette proportion diminue dans la carcasse avec SEP, SMC et SEC. Le Mn est liposoluble avec SMC et SEC (K_{ow} = 41,5 et 30,8). Le Mn est excrété par la vésicule biliaire et les chélates liposolubles de ce métal pourraient être excrétés plus rapidement. Enfin, l'effet de PEX, SEP, SMC et SEC sur la bioconcentration de Mn est l'inverse de celui observé avec Hg, Ni, Cd et Pb; l'accumulation de ces derniers est augmentée, et parfois de façon très importante, en présence de ces agents complexants.

ABSTRACT

The effects of humic and fulvic acids, potassium ethylxanthate (PEX), sodium diethyldithiophosphate (SEP), sodium dimethyl- and diethyldithiocarbamate (SMC and SEC) on the bioconcentration and the distribution of $^{54}Mn^{2+}$ in Salmo trutta were studied. Groups of 7 fish were exposed one week to 0.1 μg Mn·L^{-1} (1 μCi·L^{-1}) with and without chelating agents. Five fish per group were dissected and radioactivity in organs and tissues have been determined by impulse counting. The two other fish were used for whole body autoradiography. Octanol-water partition coefficients of Mn with and without chelating agents were also determined. Fish exposed to Mn only accumulated 0.73 ± 0.04 ng Mn·g^{-1}. Humic and fulvic acids had little effect on the bioconcentration and the distribution of Mn. The other chelating agents decreased bioconcentration by 40%. The Mn distribution was also affected; the percent body burden of viscera increased while it decreased in carcass with SEP, SMC and SEC. Mn was found liposoluble with SMC and SEC (K_{ow} = 41.5 and 30.8). It is known that Mn is excreted by bile and liposoluble metal chelates may be excreted at a higher rate. Finally, the effect of PEX, SEP, SMC and SEC on the Mn bioconcentration was the inverse of the effect observed with Hg, Ni, Cd and Pb; the accumulation of the latters was increased in presence of those chelating agents.

INTRODUCTION

Les agents complexants dissous peuvent influencer la toxicité des métaux présents dans le milieu aquatique. Les acides humique et fulvique, qui donnent une teinte jaunâtre à l'eau des cours d'eau des régions boisées, sont des agents complexants naturels pouvant complexer les métaux en solution et diminuer leur toxicité (Sprague, 1985). Certains agents complexants d'origine anthropogénique forment des chélates lipophiles avec les métaux. Dans cette catégorie, on retrouve entre autres les dithiocarbamates, largement utilisés comme pesticides et comme accélérateur dans l'industrie du caoutchouc et des plastiques, les xanthates et les dialkyldithiophosphates, surtout utilisés comme agents de flottaison dans l'industrie minière (Thorn et Ludwig, 1962; Rao, 1971; Chadwick et Watt, 1961; Fig. 1). Les sels de potassium et de sodium des ces substances sont solubles dans l'eau et peuvent former des chélates lipophiles avec les métaux dissous, affectant ainsi l'accumulation de ces derniers par la truite brune (Tjälve et Gottofrey, 1991).

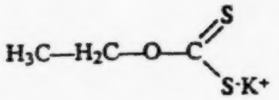
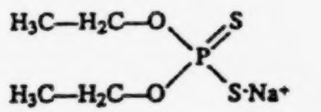
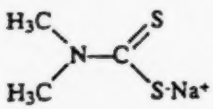
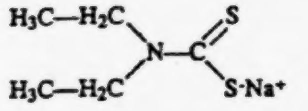
 Éthylxanthate de potassium (PEX)	 Diéthyldithiophosphate de sodium (SEP)
 Diméthyldithiocarbamate de sodium (SMC)	 Diéthyldithiocarbamate de sodium (SEC)

Fig. 1: Structure de l'éthylxanthate de potassium (PEX), du diéthyldithiophosphate de sodium (SEP), du diméthyldithiocarbamate de sodium (SMC) et du diéthyldithiocarbamate de sodium (SEC).

Le manganèse est un élément essentiel à la vie que l'on retrouve à des concentrations variant de moins de 1 $\mu g \cdot L^{-1}$ à plusieurs centaines dans le milieu aquatique (Langlois *et al*, 1983). Des concentrations élevées de ce métal dans l'eau peuvent perturber le métabolisme des poissons exposés (Cossarini-Dunier *et al*, 1988; Reader et Morris, 1988; Reader *et al*, 1988, 1989; Gonzalez *et al*, 1990), mais il n'existe pas de travaux sur la bioconcentration et la distribution du manganèse chez *Salmo trutta* à notre connaissance.

Dans ce travail, nous avons étudié la bioconcentration et la distribution de $^{54}Mn^{2+}$ chez *Salmo trutta* ainsi que les effets de six agents complexants: l'acide humique, l'acide fulvique, l'éthylxanthate de potassium (PEX), le diéthyldithiophosphate de sodium (SEP), le diméthyldithiocarbamate de sodium (SMC) et le diéthyldithiocarbamate de sodium (SEC).

MATÉRIEL ET MÉTHODES

Les truites brunes (*Salmo trutta*), agées de 1 an et pesant de 6 à 9 g, ont été founies par la pisciculture Kristforsen de Skellefteå (Suède). Le manganèse radioactif ($^{54}MnCl_2$, activité spécifique > 200 mCi·mg^{-1}) a été acheté chez Amersham. PEX, SEP, SMC et SEC ont été achetés chez ICN Pharmaceuticals tandis que l'acide humique provenait de chez Aldrich. L'acide fulvique a été obtenu par dialyse de 2 g d'acide humique et lyophilisation du dialysat. Les poissons ont été gardés dans des aquariums de verre contenant 20 L d'eau à pH 7 (0,15 g $KHCO_3$, 1,42 g $CaCO_3$, 0,16 g MgO, 4 mL de HCl 1N et 6,5 mL de H_2SO_4 1N par 100 L d'eau doublement déionisée). La température était de 11°C.

Des groupes de 7 poissons ont été exposés pendant une semaine à 0,1 μg Mn·L^{-1} (1 μCi·L^{-1}) avec ou sans agents complexants. La concentration d'acide humique était de 5 mg·L^{-1} tandis que celle de l'acide fulvique, 0,63 mg·L^{-1}, était en proportion du rendement de la dialyse (12,6%). Les quatre autres agents complexants ont été ajoutés à l'eau dans un rapport molaire de 25:1 avec le manganèse. L'eau des aquariums a été renouvelée après trois jours. Des vérifications périodiques de la concentration de ^{54}Mn dans l'eau et du pH n'ont pas permis de détecter de variations de ces paramètres au cours de l'expérience. A la fin de la période d'exposition, les poissons ont été brièvement rincés à l'eau non-radioactive et congelés à -20°C. Cinq poissons par groupe ont été disséqués et la radioactivité de tous les organes et tissus prélevés a été mesurée à l'aide d'un compteur gamma. Toutes les données ont été corrigées pour le bruit de fond et la désintégration de l'isotope et converties en ng de manganèse. Les deux autres poissons ont été sectionnés sagitallement à 20 μm et autoradiographiés selon la méthode de Ullberg (1977).

Le coefficient de partition octanol-eau du manganèse avec ou sans agents complexants a aussi été mesuré. Deux mL d'octanol-1 ont été ajoutés à 2 mL de tampon Tris-HCl (pH 7,4) contenant 0,5 μmol Mn·mL^{-1} (0,25 μCi·mL^{-1}). Dans le cas de l'acide humique et de l'acide fulvique, la concentration de Mn était de 0,005 μmol·mL^{-1}. PEX, SEP, SMC et SEC ont été ajoutés à raison de 50 μmol·mL^{-1}. L'acide fulvique à été ajouté à raison de 0,5 μmol·mL^{-1}, en assumant une masse molaire moyenne de 2000 g·mol^{-1}, tandis que la concentration d'acide humique était en proportion du rendement de la dialyse (7,9 mg·mL^{-1}). Le tout a été agité pendant 30 minutes et laissé à équilibrer pendant 4 jours à 11°C. Après équilibration, la radioactivité des deux phases a été mesurée et le coefficient de partition a été considéré comme étant le rapport de la radioactivité de la phase organique sur celle de la phase aqueuse.

RÉSULTATS ET DISCUSSION

Les truites exposées au manganèse seul pendant une semaine ont accumulé 0,73 ± 0,04 ng Mn·g^{-1}. Dans les mêmes conditions expérimentales, la truite brune accumule trois fois moins de nickel (0,28 ng Ni·g^{-1}; Tjälve *et al*, 1988), près de 7 fois plus de mercure (4,8 ng Hg·g^{-1}; Borg *et al*, 1988) et 11 fois plus de cadmium (8,6 ng Cd·g^{-1}; Tjälve et Gottofrey, 1986). C'est le foie qui a la concentration de manganèse la plus élevée (Tableau 1), suivi des viscères et des branchies, du rein et de la carcasse (reste de la tête, peau, os, nageoires), et du mucus. Les

	Concentration de manganèse (ng Mn·g^{-1} humide, X ± S.E., n = 5)						
	Groupe A (^{54}Mn^{2+} seul)	Groupe B (+ acide humique)	Groupe C (+ acide fulvique)	Groupe D (+ PEX)	Groupe E (+ SEP)	Groupe F (+ SMC)	Groupe G (+ SEC)
Branchies	2,72 ± 0,19	3,20 ± 0,27	2,96 ± 0,26	1,55 ± 0,12**	1,77 ± 0,11**	1,07 ± 0,07***	1,48 ± 0,10***
Rein	1,01 ± 0,13	1,21 ± 0,06	1,13 ± 0,07	0,66 ± 0,09	0,73 ± 0,07	0,54 ± 0,07	0,60 ± 0,07
Foie	4,74 ± 0,38	5,44 ± 0,49	4,89 ± 0,63	2,83 ± 0,38**	3,14 ± 0,28*	2,39 ± 0,20**	2,34 ± 0,16**
Viscères^{a}	3.08 ± 0,38	3,17 ± 0,55	3,09 ± 0,30	1,96 ± 0,09*	2,17 ± 0,10	2,56 ± 0,42	2,06 ± 0,34
Muscle	0,09 ± 0,01	0,13 ± 0,01*	0,12 ± 0,01*	0,07 ± 0,01	0,06 ± 0,01**	0,05 ± 0,01***	0,06 ± 0,01**
Cerveau	0,14 ± 0,02	0,20 ± 0,02	0,20 ± 0,01	0,11 ± 0,02	0,13 ± 0,01	0,10 ± 0,02	0,08 ± 0,01*
Yeux	0,26 ± 0,01	0,34 ± 0,03	0,27 ± 0,02	0,17 ± 0,02**	0,15 ± 0,01***	0,13 ± 0,01***	0,13 ±0,01***
Reste de la tête^{b}	1,11 ±0,09	1,02 ± 0,06	0,96 ± 0,09	0,60 ± 0,04**	0,59 ± 0,03**	0,46 ± 0,04***	0,53 ± 0,03**
Peau, os, nageoires	0,83 ± 0,05	0,77 ± 0,06	0,67 ± 0,05*	0,47 ± 0,05***	0,42 ± 0,03***	0,37 ± 0,04***	0,40 ± 0,03***
Mucus	0,64 ± 0,09	0,51 ± 0,06	0,46 ± 0,05	0,23 ± 0,01**	0,21 ± 0,01**	0,21 ± 0,02**	0,21 ± 0,01**
Corps entier	0,73 ± 0,04	0,75 ± 0,04	0,69 ± 0,05	0,42 ± 0,03***	0,44 ± 0,02**	0,38 ± 0,04***	0,40 ± 0,03***

Tableau 1: Concentration de manganèse dans les organes et tissus de truites brunes exposées pendant 1 semaine à ^{54}Mn^{2+} (0,1 μg Mn·L^{-1}) en présence ou en l'absence d'agents complexants. La concentration d'acide humique était de 5 mg·L^{-1}, celle d'acide fulvique de 0,63 mg·L^{-1} et celle de PEX, SEP, SMC et SEC dans un rapport molaire de 25:1 avec la concentration de manganèse.

* significativement différent du groupe A, $p < 0{,}05$

** significativement différent du groupe A, $p < 0{,}01$

*** significativement différent du groupe A, $p < 0{,}001$

[a] Viscères sans le rein et le foie

[b] Tête sans les yeux, le cerveau et les branchies

	Pourcentage du contenu total en manganèse (X ± S.E., n = 5)						
	Groupe A ($^{54}Mn^{2+}$ seul)	Groupe B (+ acide humique)	Groupe C (+ acide fulvique)	Groupe D (+ PEX)	Groupe E (+ SEP)	Groupe F (+ SMC)	Groupe G (+ SEC)
Branchies	15,03 ± 0,65	18,63 ± 0,63**	17,54 ± 0,43*	14,66 ± 1,27	15,09 ± 0,66	10,43 ± 0,60	13,58 ± 0,82
Rein	2,10 ± 0,16	2,11 ± 0,17	1,90 ± 0,07	1,73 ± 0,12	2,10 ± 0,10	1,82 ± 0,06	2,00 ± 0,14
Foie	7,38 ± 0,84	7,97 ± 0,76	7,43 ± 0,84	6,50 ± 0,62	6,64 ± 0,58	5,70 ± 0,58	6,55 ± 0,33
Viscères^{a}	24,82 ± 1,32	22,74 ± 0,64	24,99 ± 0,93	26,39 ± 0,67	31,08 ± 0,75**	38,77 ± 2,46**	31,97 ± 2,68*
Muscle	5,95 ± 0,27	8,19 ± 0,31***	8,54 ± 0,70*	6,50 ± 0,62	6,64 ± 0,58	5,70 ± 0,58	6,55 ± 0,33
Cerveau	0,17 ± 0,03	0,26 ± 0,04	0,26 ± 0,03	0,22 ± 0,03	0,26 ± 0,03*	0,31 ± 0,07	0,20 ± 0,02
Yeux	1,13 ± 0,13	1,46 ± 0,18	1,21 ± 0,09	1,12 ± 0,03	0,96 ± 0,07	1,04 ± 0,09	0,94 ± 0,04
Reste de la tête^{b}	17,50 ± 1,44	15,76 ± 0,74	15,25 ± 0,71	15,66 ± 0,67	13,84 ± 0,92	12,80 ± 0,50*	14,19 ± 0,91
Peau, os, nageoires	24,53 ± 0,71	20,97 ± 0,81*	21,43 ± 0,74*	24,06 ± 0,51	21,19 ± 0,94*	20,65 ± 0,99*	21,76 ± 0,91*
Mucus	1,42 ± 0,19	1,99 ± 0,23	1,51 ± 0,16	2,48 ± 0,16**	2,02 ± 0,15*	2,64 ± 0,15**	2,14 ± 0,15*

Tableau 2: Pourcentage du contenu total en manganèse des organes et tissus de truites brunes exposées pendant 1 semaine à $^{54}Mn^{2+}$ (0,1 $\mu g \; Mn \cdot L^{-1}$) en présence ou en l'absence d'agents complexants. La concentration d'acide humique était de 5 $mg \cdot L^{-1}$, celle d'acide fulvique de 0,63 $mg \cdot L^{-1}$ et celle de PEX, SEP, SMC et SEC dans un rapport molaire de 25:1 avec la concentration de manganèse.

* significativement différent du groupe A, $p < 0,05$

** significativement différent du groupe A, $p < 0,01$

*** significativement différent du groupe A, $p < 0,001$

[a] Viscères sans le rein et le foie

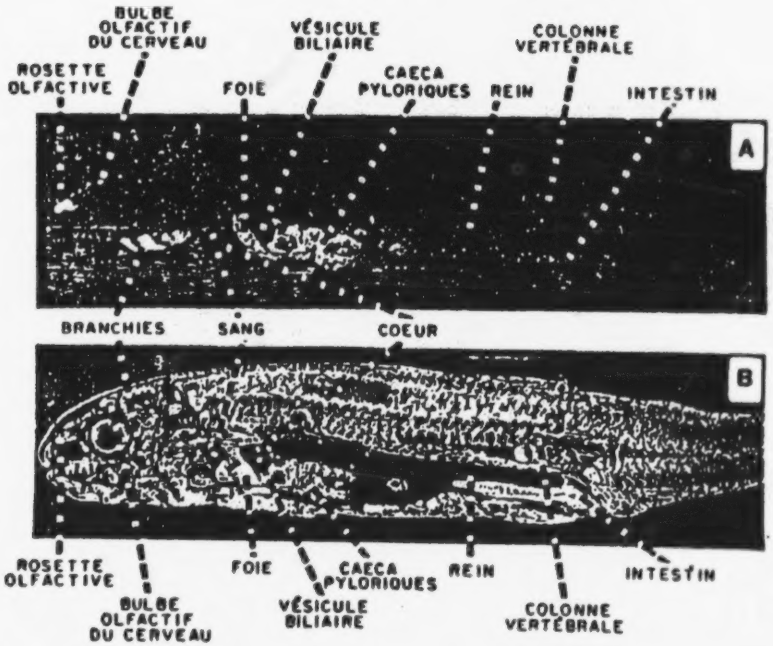
[b] Tête sans les yeux, le cerveau et les branchies

concentrations les plus faibles sont retrouvées dans les yeux, le cerveau et le muscle. C'est la carcasse qui contient la plus grande proportion du contenu total en manganèse (Tableau 2), soit 42%. Les viscères et les branchies contiennent aussi une proportion importante du manganèse, suivis du foie, du muscle, du rein, du mucus, des yeux et du cerveau. Les autoradiogrammes confirment cette distribution (Fig. 2). Ils montrent de plus la présence de manganèse dans les os et le système olfactif (rosette olfactive, nerf olfactif et bulbe olfactif du cerveau, fig. 3) et son absence dans le sang (Fig. 2).

Les contaminants présents dans le milieu aquatique pénètrent dans l'organisme des poissons par les branchies pour être ensuite distribués à l'ensemble de l'organisme par le sang. Lydén *et al* (1983) ont démontré que le manganèse injecté par voie intraveineuse chez la souris disparaît très rapidement de la circulation sanguine pour être distribué à l'ensemble de l'organisme, ce qui pourrait expliquer l'absence de radioactivité dans le sang de la truite. Au niveau cellulaire, le manganèse se concentre dans les mitochondries et on le retrouve donc surtout dans les organes riches en mitochondries, comme le foie et le rein (Leach et Lilburn, 1978), comme c'est le cas ici pour la truite. La concentration relativement élevée de manganèse dans les intestins pourrait s'expliquer par le fait que le manganèse est presque exclusivement excrété par voie biliaire dans les intestins, et de là par les fèces (Papavasiliou *et al*, 1966; Šarić, 1986). La concentration de manganèse dans les os d'un poisson comme *Catostomus commersoni* est un ordre de grandeur plus élevée que dans le foie (Bendell-Young et Harvey, 1986). Cependant, Pentreath (1973) a démontré que la vitesse d'accumulation du manganèse dans le squelette est très lente chez la plie *Pleuronectes platessa* L. . Le [illegible] phénomène semble se produire chez *Salmo trutta* puisque que les autoradiogrammes montrent qu'il y a moins de radioactivité dans les os comparativement au foie. L'accumulation de [illegible] dans le système olfactif de *Salmo trutta* a déjà été démontré pour le cadmium et le mercure (Tjälve et Gottofrey, 1986; Gottofrey et Tjälve, 1991).

Les acides humique et fulvique n'ont que peu d'effets sur la bioconcentration et la distribution du manganèse chez la truite brune (Tableau 1 et 2). La concentration de manganèse est plus élevée dans le muscle. Ce dernier compte également pour une plus grande part du contenu total en manganèse. La proportion de manganèse contenu dans les branchies est un peu plus élevée que pour le groupe exposé au manganèse seul tandis qu'elle diminue dans la peau, les os et les nageoires. Il n'est pas possible d'établir la signification de ces différences, petites mais significatives, avec les présentes données.

PEX, SEP, SMC et SEC diminuent de 40 à 45% le contenu total en manganèse des truites. En général, la concentration de manganèse diminue significativement dans tous les organes et tissus, à l'exception des viscères (sauf pour PEX) et du cerveau (sauf pour SEC). PEX et SEP ne diminuent pas la concentration de manganèse dans le rein de façon significative. C'est le SMC qui semble avoir l'effet le plus marqué. PEX n'a que peu d'effets sur la distribution du manganèse si ce n'est une légère augmentation de la proportion du manganèse contenue dans le muscle et le mucus. Les trois autres agents complexants ont comme principal effet d'augmenter la proportion de manganèse dans les viscères et de la diminuer dans la peau, les os et les nageoires. La proportion de manganèse est aussi plus faible dans le reste de la tête pour SMC. Cette augmentation de la proportion de manganèse contenu dans les viscères pourrait être le signe d'une augmentation de l'excrétion du manganèse. Les coefficients de partition octanol-eau


BULBE
OLFACTIF
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COLONNE
VERTÉBRALE
INTESTIN
A
BRANCHIES
SANG
COEUR
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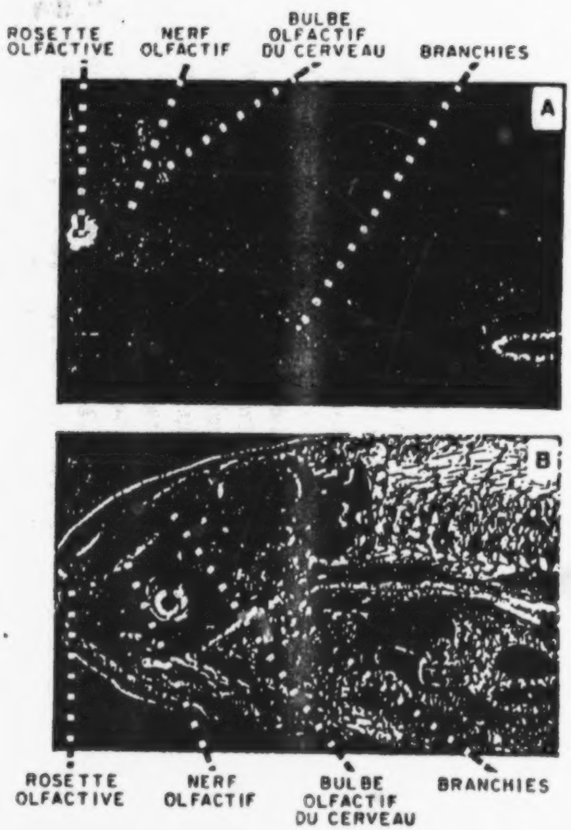
ROSETTE
OLFACTIVE
BULBE
OLFACTIF
DU CERVEAU
FOIE
VÉSICULE
BILIAIRE
CAECA
PYLORIQUES
REIN
COLONNE
VERTÉBRALE
INTESTIN

Fig. 2: (A) Macroautoradiogramme d'une truite brune (Salmo trutta) exposée pendant une semaine à 0,1 $\mu g \ Mn \cdot L^{-1}$. (B) Section correspondante.


ROSETTE
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BRANCHIES
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DU CERVEAU
BRANCHIES

Fig. 3: (A) Détail d'un macroautoradiogramme montrant la distribution du manganèse dans le système olfactif d'une truite brune (Salmo trutta) exposée pendant une semaine à 0,1 $\mu g \ Mn \cdot L^{-1}$. (B) Section correspondante.

	Coefficient de partition	% Mn^{2+} dans la phase organique
Mn^{2+} seul	< 0,001	< 0,1
+ acide humique	0,0028 ± 0,0002	0,3
+ acide fulvique	0,0107 ± 0,0003	1,1
+ PEX	0,014 ± 0,001	1,4
+ SEP	0,019 ± 0,001	1,9
+ SMC	41,5 ± 3,4	97,6
+ SEC	30,8 ± 7,9	96,9

Tableau 3: Coefficient de partition octanol-eau du manganèse en présence d'agents complexants. Le coefficient de partition est défini comme le rapport de la radioactivité de la phase organique sur celle de la phase aqueuse (X ± S.E., n = 5). Voir la section matériel et méthodes pour les conditions expérimentales.

du manganèse montrent que le manganèse est liposoluble en présence de SMC et SEP (Tableau 3). Les chélates liposolubles du manganèse pourraient ainsi être excrétés plus rapidement par voie biliaire. Les autoradiogrammes montrent pourtant qu'il n'y a pas de manganèse dans la vésicule biliaire. Il semble cependant que le temps de résidence du manganèse dans la bile soit très court puisque la concentration de manganèse dans la bile diminue aussi rapidement que dans le sang (Cotzias, 1958).

Ces données soulèvent cependant plusieurs interrogations; 1° pourquoi SEP qui n'a que peu d'effets sur la liposolubilité du manganèse modifie-t-il sa distribution comme SMC et SEC, 2° pourquoi PEX qui ne change pas beaucoup la liposolubilité du manganèse lui aussi et ne modifie pas sa distribution diminue-t-il l'accumulation de manganèse, 3° pourquoi PEX, SEP, SMC et SEC diminuent-ils l'accumulation de manganèse alors qu'ils provoquent une augmentation, parfois importante, de l'accumulation du cadmium (Gottofrey et al, 1988a), du nickel (Gottofrey et al, 1988b), du mercure et du méthylmercure (Gottofrey et Tjälve, 1991) et du plomb (Gottofrey et Tjälve, données non-publiées) ? Bien que la lipophilicité puisse être un facteur significatif influençant l'accumulation et la distribution, la biodisponibilité et la stabilité du chélate sont aussi des facteurs très importants. Des expériences supplémentaires seront nécessaires afin de déterminer quel est le facteur prépondérant ou la combinaison de facteurs caractérisant l'influence de ces agents complexants sur la bioconcentration et la distribution du manganèse chez Salmo trutta.

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ANALYSIS OF TRIALKYLTIN IN ESTUARINE SEDIMENTS AND OYSTER TISSUES.

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INTRODUCTION

A method which was relatively quick, inexpensive and accurate and capable of being carried out by a large number of organizations was needed for the routine analysis of tributyltin in oyster tissues and sediments. In this case, the method was developed for the analysis of TBT, total copper, zinc, lead and tin in tissues of individual oysters. It develops further the analytical procedures of McKie (1987) which would seem to be one of the better methods published thus far, utilizing the carbon furnace technique in the analysis of TBT compounds. This particular method analyses for the whole trialkyltin group and assumes that this will mainly consist of the tributyltin species. The adaptation of a method which can measure each of the above contaminants in an individual oyster gives potentially far more useful data than does the analysis of homogenates of numbers of these individuals.

METHODS AND MATERIALS

When thawed oysters were opened with a stainless steel blade, the adductor muscle was severed from both valves with a stainless steel scalpel and the entire contents of each oyster then shucked into a 100 ml acid washed Pyrex beaker and accurately weighed.

The oyster was then transferred to a 500 ml conical flask by rinsing the beaker with 50 ml of hydrochloric acid. The mouth of the flask was covered with Whatman parafilm.

The tissue was digested, at room temperature, over 2-3 hours in the flask which was shaken slowly by a mechanical shaker. 100 mls of AR n-hexane was then added to the flask and shaken for a further hour. This mixture was allowed to stand for one hour to separate into distinct layers.

Metal analysis
The lower acid layer was then transferred into a 150 ml beaker to which 10 mls of AR nitric acid was added. The mixture was covered with a watch glass and digested on a hot plate at 70' C, for approximately one hour, to give a clear digest. The solution was filtered through a Whatman 541 filter paper using a 1M nitric acid wash. The filtrate was made up to 200 mls volumetrically using reverse osmosis water.

In addition to the oyster samples four blanks were produced following the above procedure but omitting the sample addition. Each oyster solution was analyzed for total copper, zinc, lead and tin on a Labtam Plasmascan model 8410 Inductively Coupled

Plasma spectrophotometer (ICP). Standards being made up with RO water in a matrix approximating that of the sample solutions, ie 25% v/v hydrochloric and 5% v/v nitric acids. Analyte concentrations were produced so as to give suitable standard curves. The composition of these standards is given in Table 1

Table 1. Metal concentration in each standard utilized in the calibration of the ICP.

Metal	Standard 1 ppm	Standard 2 ppm	Standard 3 ppm
copper	1	5	10
zinc	1	10	50
tin	1	10	20
lead	1	5	10

Final counts on the ICP were the average of three 10 second integrations and were accepted only if the relative standard deviation was below 1.0%

Trialkyltin analysis

The n-hexane layer in the conical flask (where digestion occurred) contains the tributyltin chloride which had been extracted from the acid digest. To this solution 130 mls of 3% w/v sodium hydroxide was added and the flask again covered with parafilm, before being placed on the mechanical shaker for 1 hour at low speed. The mono- and dibutyltins are extracted from the solution into the caustic layer, thereby leaving only trialkyltin in the organic layer. The mixture was allowed to stand for 4-6 hours at ambient temperature, to give distinctive layers. The caustic layer is then discarded as the method described herein is only concerned with TBT analysis.

Fifty mls of the upper n-hexane layer was bulb pipetted into a 100 ml flask, to which 5 mls of AR nitric acid was added. The n-hexane was then evaporated on a rotary evaporator at 40° C to a final volume of 4-5 mls.

The acid fraction is cooled and quantitatively transferred to a 25 ml volumetric flask. Prior to make up, 15 mls of 25% w/v ammonium dihydrogen orthophosphate was added to the flask to act as a modifier (graphite furnace). All samples were run on a Varian Spectra 20 Graphite Furnace atomic absorption spectrophotometer (GFAAS) for elemental tin. Samples were measured immediately after processing and run against tin standards freshly prepared for each run. Four blanks and three spiked homogeneous samples were analyzed as described above. Blanks were produced as before except that the sample addition step was omitted. Spiked solutions had a range of TBT-chloride added to the initial acid step, again omitting sample addition. These were equivalent to the addition of 0.0, 1.39, 3.46, and 6.93 µg of tin. These samples were also treated as per the above method. Spiked samples were run to give a range of results representing the recovery efficiency for the method.

All applications were carried out in triplicate using the automatic sampler attached to the Spectra 20. This was programmed to deliver triplicate 20 µL injections of premixed samples, blanks and standards.

Sediment Analysis

Triplicate 20 g subsamples from each site were treated in the same way as the oyster tissues discussed above. From the initial graphite furnace readings it was found that the levels of TBT in the sediments were much higher than those detected in the oyster tissues. For this reason subsequent TBT levels were measured by ICP in preference to GFAAS.

RESULTS and DISCUSSION

For TBT in tissues (Table 2), zinc (Table 3) and copper (Table 4),the variances between individuals is quite large as shown by the confidence limits and the standard error. In some cases the high variance may be attributed to the measurement of the analyte being taken close to the detection limits of the method, *ie* copper levels below 3 µg/g (Table 4).

TBT levels were measured as elemental tin and ranged from 0.0-157.5 ng of tin per g of wet oyster tissue. The highest reading being at site AR in Merimbula Lake. The absence of permanently moored boats in this lake and its shallow depth should mean a faster microbial and photodegradation of the various TBT compounds (Seligman *et al.*, 1988) It would seem that a combination of low flush rates such as in some bays and upstream areas, together with a large number of permanently moored boats could be major factors resulting in the higher levels of TBT contamination (Batley, 1988). Another factor effecting TBT concentration could also be the presence of a suitable substrate with the ability to act as a type of "sink", so that TBT compounds or breakdown products can be reintroduced into the water column over an extended period.

The results for the Georges River are to be expected due to the large numbers of moored boats in the estuary. Wallis Lakes has far less boat use compared with the Georges River in metropolitan Sydney, and these results reflect this condition. The value for site WLCL is consistent with increased boat use at this site, which is a catching lease 10 m from a permanently moored fishing fleet.

Zinc levels in Wallis Lakes and Merimbula Lakes system were on the whole lower than those of the Georges River sites. The levels of copper in the Georges River (Table 4) can be seen to be of a greater magnitude than those in the Wallis Lakes and Merimbula Lakes. None of the sites analyzed were found to be above the recommended levels for copper in shellfish as stated by the National Health and Medical Research Council (NHMRC)(70 µg/g wet weight). Basically though these levels of zinc (and for copper) in these cultivated oysters were in the same order of magnitude as has been reported previously for Georges River (Mackay *et al.*, 1975).

It is postulated that the primary sources of zinc were galvanized building materials, sacrificial electrodes, sewage and storm water runoff from the surrounding urban areas and outboard motors.

Table 2. TBT levels in tissues from individual oysters, 5 individuals per site.

Site	Mean µg/g	95% Confidence limits	Standard error
Georges River			
CB	0.0	-	
TP	0.0	-	
WB	12.8	-12.15- 37.75	8.99
CB	41.2	-73.14-206.44	41.19
HB	0.0	-	-
WR	40.4	-71.83-152.63	40.43
BB	76.0	40.12-111.88	12.90
LKB	9.4	-6.62- 25.42	5.77
Wallis Lakes			
RAP	0.0	-	-
MI	0.0	-	-
WLCL	10.5	-122.47-143.47	10.47
Merimbula Lake			
ML	32.2	-7.16-71.76	12.40
AR	157.5	-72.72-387.72	72.35

Table 3. Zinc levels in tissues from individual oysters, 5 individuals per site.

Site	Mean µg/g	95% Confidence limits	Standard error
Georges River			
CB	266	248-284	6.6
TP	241	195-287	16.6
WB	289	224-354	23.3
CB	255	214-296	14.6
HB	326	287-365	14.0
WR	292	195-389	34.9
BB	369	265-473	37.6
LKB	527	493-561	12.3
Wallis Lakes			
RAP	232	138-326	33.8
MI	205	103-304	35.7
WLCL	207	180-595	30.5
Merimbula Lake			
ML	135	45-225	32.5
AR	360	236-484	44.8

Table 4. Copper levels in tissues from individual oysters, 5 individuals per site.

Site	Mean µg/g	95% Confidence limits	Standard error
Georges River			
CB	3.6	2.20-5.08	0.52
TP	17.1	2.41-31.75	5.29
WB	36.5	27.31-45.61	3.30
CB	23.7	6.14-41.22	6.32
HB	33.0	24.23-41.77	3.16
WR	33.0	25.74-40.22	2.61
BB	55.4	41.03-69.85	5.19
LKB	62.4	45.86-78.90	5.95
Wallis Lakes			
RAP	3.8	2.01-5.51	0.63
MI	0.0	-	-
WLCL	13.8	30.67-58.27	3.50
Merimbula Lake			
ML	2.8	4.94-10.50	2.78
AR	0.7	1.25-2.65	0.70

CONCLUSION

The method described has a recovery of between 95 and 110% with a detection limit of 4 ng/g of Sn (wet weight) in both oyster tissues and sediments. The method is faster and less consuming of resources than the more common method utilizing Gas Liquid Chromatography/electron capture, an important consideration for large monitoring programmes.

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STUDY OF COPPER AND ZINC LEVELS IN ESTUARIES USING THE SYDNEY ROCK OYSTER AS A BIOMONITOR.

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INTRODUCTION

The use of benthic organisms to monitor heavy metals in the environment has several advantages over simply measuring concentrations in water and/or sediments. Various species of oysters and mussels are believed to bioaccumulate high concentrations of metals and probably to bio-regulate the same.

The problems of using the water body and sediments as "biomonitoring agents " and the advantages of using shellfish have been discussed by Phillips (1977, 1978) and Farrington (1983) amongst others.

In this project, the objectives are to

- evaluate the Sydney rock oyster as a biomonitor
- assess uptake in field populations
- correlate metal levels in the oyster and sediment
- compare sites within an estuaries.

By necessicity, the project has taken the format of an elaborate pilot study.

Three estuaries were chosen for examination because of their importance to oyster production in New South Wales and include the three largest oyster producing areas; they being Port Stephens/Karuah River; Wallis Lake and Georges River/Botany Bay. A smaller system was also included. The catchment of Georges River/Botany Bay can be described as urban, industrialized, of Port Stephens/Karuah and Wallis Lake as agricultural, forest and national park and in part recreational, and that of Merimbula Lake as forest, national park.

MATERIALS AND METHODS

Within each area, oysters were sampled initially from commercially grown stocks or wild oysters growing on rocks within the mangroves, but all were sampled from intertidal areas. Samples were collected, frozen and taken back to the laboratory for analysis using AAS. The various variables are discussed below.

RESULTS AND DISCUSSION

A brief synopsis of our findings serve to illustrate the problems and the continuing direction of our programme and perhaps point to areas which have been ignored or assumed in many other studies including Mussel Watch Programmes. In this brief review, the Georges River/Botany Bay results are examined in more detail for they present an overall impression of the processes apparently occurring in eastern Australian waters.

(a) Wild vs cultivated oysters

Superficially it would appear that there is a major difference between metal levels in cultivated oysters and those in indigenous or wild ("feral") growing oysters. This is especially noticeable for zinc levels. However caution must be exercised because data was confounded by a time factor. For even though metal levels are much higher in wild populations compared with cultivated oysters for all sites, a temporal as well as a methodological difference may be influencing the results when the 1987 data are compared with that of 1975. But as a preliminary finding, its value must be tempered by this time difference, ie there could be a real difference between growing conditions, or the difference could be due to thew 1975 vs 1987 factor.

In 1990/91 when cultivated oysters were compared to wild oysters from the Georges River, there was no significant differences between the oysters for copper and zinc loadings despite the apparent differences. The results were for copper: 30-171 µg/g cultivated *vs* 49-78 µg/g wild; for zinc, 470-1580 µg/g cultivated *vs* 570-880 µg/g wild. The levels of copper and zinc in the Georges River were found to be higher in indigenously growing oysters compared with the cultivated oysters. Further the indigenous or wild grown oysters had levels of metal which often exceeded the recommended NHMRC health standards for shellfish.

(b) Variation between samples: samples from single oysters vs samples from pooled oysters.

Copper and zinc were both measured as µg/g wet weight of tissue. Although these results for copper and zinc are of similar magnitude to previous levels found by Mackay *et al.* (1975), nevertheless there was a marked difference between the levels found in the mean derived from the analysis five separate individual samples compared with the means of subsamples derived from the analysis of the homogenized 10 or 20 animals from the same sample site. This variability needs to be resolved by further analysis.

(c) Variability between systems

The levels of copper and zinc as µg/g wet weight of oyster tissue in the Georges River are much greater than those from Wallis Lake and Merimbula Lake. There was one site at Merimbula Lake, which had the third largest reading of zinc (360 µg/g wet weight) and may reflect runoff from an adjacent small airport. Like the site in the Georges River with the largest zinc value (527 µg/g), these results may be a reflection of leacheates from fill material used in construction of the site. The results from cultivated oysters in this comparison were all within NHMRC limits.

(d) Variability within systems

A definite correlation between copper concentrations and distance from the mouth of an estuary was demonstrated. There was no correlation between zinc concentrations in oyster tissue and distance, although there was a correlation between zinc and copper concentrations in the sediments from the same sites as the oysters with distance to the mouth of the estuary.

(e) Distribution of metals within the oyster

When the mass of oyster tissue was expressed as a proportion of total mass and compared with the amount of metal in that tissue expressed as a proportion of total metal level in the whole oyster, it was found that in general, gill and mantle had greater concentrations than did the adductor muscles. But depending upon whether the oyster was from a stressed (=high metal loads) or non-stressed site, so the relationship changed.

From areas of low metal concentration, it was found that the concentrations of copper and zinc were approximately in the same proportion as the proportion of the tissue types, the exception being that there was a lower concentration in the adductor muscle and more in the labial palp.

From areas of high metal concentration, there was a greater concentration of copper and zinc in the labial palps and gonads, compared with the expected based upon the proportion of tissues. The mass of gonad and palps was much lower compared with that in the non-stressed oysters. Uptake experiments may elicit the true relationship here.

CONCLUSIONS

Evaluation of available information on bioaccumulation of heavy metals in oysters and mussels indicates that although there are considerable data available in the literature, there are significant unexplained gaps, or areas avoided, as has been implied herein, certainly this is the case for the Sydney rock oyster, *Saccostrea commercialis* and to estuarine areas of eastern Australia. Because of the documented information in industrialized urban areas and the important biological resources of the Georges River/Botany Bay, it is recommended that studies of bioaccumulation be included in future biomonitoring programmes, as follows:

- bioaccumulation studies to provide the necessary information regarding accumulation/regulatory rates need to be determined by establishing growing colonies which can be routinely sampled over time and space, for a significant component of the species lifespan;
- apart from determining heavy metal levels in the whole animal, tissue analyses of indigenous mussels as well as the local oyster species may provide useful indicators of the temporal and spatial patterns of contamination by both heavy metals and organic compounds;
- because of the limited available data, the relationship between metal levels found in commercial and neighboring "wild" oysters should be determined;
- species should be evaluated from other industrialized and urbanized *vs* non-industrialized or urbanized catchments;
- future programmes should concentrate on a few selected species, such as the rock oyster and one or two mussel species which between them, represent the salinity ranges of the estuary;
- studies of the spatial extent of organochlorines, PCBs and PAHs contamination should be initiated, especially in areas where commercial or recreational fisheries (including oysters, prawns, crabs and fish) are present.

All these programmes are currently underway in various estuaries along the eastern coastline, using the rock oyster and three indigenous mussel species.

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LE TRANSFERT DES BIPHÉNYLS POLYCHLORÉS DES SÉDIMENTS AUX GASTÉROPODES HERBIVORES VIA LE PÉRIPHYTON.

C. Vanier et D. Planas, GEOTOP - Départmente de Biologie, Université du Québec à Montréal, Montréal, Québec.

Dans les écosystèmes aquatiques les sédiments sont considérés comme d'importants réservoirs de contaminants hydrophobes tels les biphényls polychlorés (BPC). Bien que les producteurs primaires périphytiques soient à la base du réseau trophique dans les rivières et le littoral des lacs, l'étude de l'accumulation de contaminants par le périphyton colonisant les sédiments a jusqu'ici été négligée. Exposés de façon continue aux BPC accumulés dans les sédiments, ces algues et microorganismes jouent possiblement un rôle dans la mise en disponibilité des BPC et pourraient être impliqués dans la contamination d'organismes herbivores, consommés à leur tour par des poissons, mammifères et oiseaux aquatiques. Nos hypothèses sont les suivantes: 1) le périphyton accumule des BPC lorsqu'exposé à des sédiments contaminés, 2) la contamination du périphyton est fonction de celle des sédiments qu'il colonise, et 3) le périphyton transfère sa contamination aux invertébrés herbivores.

Des substrats artificiels (filtres de Teflon; $74 \mu m$) colonisés par le périphyton au lac St-Pierre (lac fluvial du Saint-Laurent) ont été exposés en laboratoire à des sédiments contaminés au 2,2',4,4',5,5'-hexachlorobiphényl marqué au ^{14}C (HCB-^{14}C). Deux niveaux d'exposition ont été utilisés, de l'ordre de 0.3 et 2.2 mg HCB-^{14}C/kg poids sec de sédiments; un 3ième aquarium contenant des sédiments non marqués a servi de témoin. De l'eau du lac a été ajoutée aux aquariums après filtration. Après 30 jours d'exposition du périphyton aux sédiments contaminés, des gastéropodes (Physea sp) distribués dans 3 nouveaux aquariums ont été nourris de périphyton contaminé. Afin de déterminer la contamination des gastéropodes *via* la désorbtion d'HCB-^{14}C du périphyton, un pot de verre contenant des substrats contaminés et recouvert d'un filtre de teflon a été déposé dans un 4ième aquarium avec des gastéropodes nourris de périphyton non contaminé.

Nous présenterons les niveaux de contamination des 3 composantes étudiées : sédiments, périphyton et gastéropodes. Nous établirons de plus la relation entre la contamination du périphyton et celle des sédiments, de même qu'entre la contamination des gastéropodes et celle du périphyton. La contamination en HCB-^{14}C du périphyton et des gastéropodes nourris de ce périphyton, suggère qu'en milieu naturel les sédiments constituent une source importante de contaminants pour le périphyton, augmentant la biodisponibilité de ces contaminants pour les herbivores et le reste du réseau trophique.

EMERGING INSECTS AS A BIOTIC PATHWAY FOR MOVEMENT OF 2,3,7,8-TETRACHLORODIBENZOFURAN FROM LAKE SEDIMENTS.

W.L. Fairchild, D.C.G. Muir, R.S. Currie and A.L. Yarechewski, Pesticide Research Laboratory, Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, and Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba.

Four 5 m diameter, natural bottom enclosures, located in a littoral zone of an oligotrophic lake at the Experimental Lakes Area in north western Ontario, were treated with 10 µg of ^{3}H-2,3,7,8-tetrachlorodibenzofuran (TCDF). TCDF was added to sorbed to natural lake sediments. Initial whole water concentration of TCDF was 34.8 to 43.9 pg/L (4L extracted with dichloromethane), this decreased to about 2 pg/L at day 21 and remained below 2 pg/L to day 120. Extracted sediment of 0-2 cm layers had fairly constant TCDF concentrations in the 20 pg/g dry weight range. Benthic invertebrates had peak concentrations from 50-250 pg/g wet weight. Emerging insects collected in submerged funnel traps had concentrations from 100-300 pg/g wet weight. Estimated export of TCDF from epilimnetic sediments by emerging insects was from 0.6 to 2.1% of the total sediment load annually. The presence of TCDF in emerging insects indicates the potential for contaminant export from lake sediments to terrestrial food chains.

WHY ARE ST. LAWRENCE BELUGA WHALES SO CONTAMINATED?

B.E. Hickie, P.V. Hodson and M. Mackay, Department of Fisheries and Oceans, Institut Maurice-Lamontagne, Mont-Joli, Québec, and Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario.

The St. Lawrence beluga whale (Delphinapterus leucas) population has been classified as endangered since 1983. Contaminants have been implicated as an important factor contributing to this situation. As top level predators, living in excess of 30 years, belugas accumulate extraordinarily high levels of persistent contaminants (including PCBs, ΣDDT, and mirex). Contaminant burdens increase with age in mature whales, with males having consistently higher burden than females; while contaminant levels in juveniles are highly variable. Since the population is endangered, direct study of the accumulation and possible effects of contaminants are limited to the examination of stranded whales. With such limits, pharmacokinetic modelling can be an effective indirect means of gaining greater insight into the problem.

Modelling has involved adapting standard mammalian pharmacokinetic models to the attributes of the beluga, and incorporating aspects of their life-history and physiology (growth, energetics, diet, gender, reproduction). The model is being used to explore a number of hypotheses including the importance of migrating eels as a contaminant transport vector from Lake Ontario.

CONTAMINATION LEVELS OF RECENT SEDIMENTS SAMPLED IN THE UPPER SAGUENAY FJORD (Québec). E. Pelletier, B. Sainte-Marie and P. Hodson, Centre Océanographique de Rimouski, Rimouski, Québec, and Department of Fisheries and Oceans, Institut Maurice-Lamontagne, Mont-Joli, Québec.

A box core (20 x 30 cm) was sampled in May 1990 in bottom sediments of the upper section of the Saguenay Fjord (Québec) and was sub-sampled under nitrogen in a specially designed glovebox into 2-cm thick horizontal layers down to 59 cm depth. Each layer corresponds approximately to 2/3 year as estiamted from the position of the Saint-Jean-Vianney landslide deposit which occurred in May 1971. Sediment samples were analyzed for Zn, Cd, Cr, Cu and Hg by atomic adsorption. Polyaromatic hydrocarbons (PAHs) were determined by gas chromatography after liquid extraction. Cr and Cu showed little changes in their respective concentration profiles down to 59 cm whereas [Hg] increased drastically at depths corresponding to years 1981-1985. A major increase of [Zn] and [Cd] was also observed for years 1971-1976. Total PAHs increased gradually from ≈3.1 µg/g (dry weight) near the surface to 14.9 µg/g in the layer 48-50 cm. Fluoranthene, pyrene, chrysene and benzo(a)pyrene were the dominant peaks of chromatograms. Origin and behaviour of these contaminants will be discussed with respect to industrial activities (aluminum plants and paper mills) of the Chicoutimi-Alma area.

FATE OF ORGANOCHLORINES DOWNSTREAM OF AN ALBERTA BLEACHED KRAFT PULP MILL. S.M. Swanson and D.A. Birkholz, Beak Associates, Calgary, Alberta, and Envirotest Laboratories, Edmonton, Alberta.

Part of a study sponsored by Proctor & Gamble of the Wapiti/Smoky River system downstream from the P&G bleached kraft pulp mill in Grande Prairie, Alberta, focussed on the fate of organochlorines. Samples of water, sediments and fish were taken before and after mill improvements that included implementation of 70% chlorine dioxide substitution in October 1990. Data to date show that while chlorinated organics remained detectable in water 120 km downstream of the plant, the main mechanism of transport was suspended sediment. Dioxin/furan levels suspended sediment was related to total organic carbon content. Based on 1990 data, the bottom sediments do not appear to be an important source or sink for organochlorines. Bottom-feeding longnose suckers had very low to non-detectable levels of all organochlorines analyzed. Mountain whitefish, on the other hand, had higher levels. There was some indication of a decrease in organochlorine levels after the implementation of 70% chlorine dioxide substitution; however, this must be confirmed.

HOW DO WE HANDLE THE REGISTRATION OF COPPER-BASED PRODUCTS?

P.-Y. Caux, Environment Canada, Commercial Chemicals Branch, Ottawa, Ontario.

The distribution and speciation of copper in the environment is complex. The biotic and abiotic interactions are numerous and complicated by the transient nature of the environment. If one considers the number of copper-based products used as pesticides, the task of evaluating the environmental fate for all possible chemical species becomes enormous. Generally, the common xenobiotic and biologically active ingredient is the copper ion (Cu^{2+}). We are considering adopting a generic approach in the our data requirements from registrants. The compounds under consideration have a similar mechanism of action. Essentially, Cu^{2+} leaches from a fixed matrix into a surficial layer at a concentration lethal to invading organisms (algae, fungi). The most sensitive non-target biota which will come in contact with the toxicant has to be protected. For the most part, we are dealing with the protection of the aquatic and sediment life. The immediate question is, what are the total copper concentrations found in particular aquatic ecosystems subjected by constant loadings of copper-based products? Since Canadian guidelines have been derived for total copper, for aquatic life ($2\ \mu g \cdot L^{-1}$) and for sediment life ($25\ \mu g \cdot L^{-1}$), these concentrations should not be exceeded if we are to protect the environment. Specific recommendations that were given in the poster included:

1) A memo to registrants (R-Memo) defining Environment Canada's needs with regards to copper-based products may prove to be beneficial to both government and industry

2) CCB's future role in the post-registration of pesticide products will need to be assessed

3) A control is needed on release rates of Cu^{2+} from fixed matrices.

CAN CHRONIC CYANIDE EXPOSURE INDUCE CHRONIC THIOCYANATE TOXICITY IN RAINBOW TROUT?

R.P. Lanno and D.G. Dixon, Ecological Services for Planning Limited, Guelph, Ontario, and Department of Biology, University of Waterloo, Waterloo, Ontario.

Exposure of rainbow trout to waterborne cyanide (CN^-) results in the bioaccumulation of thiocyanate (SCN^-) in plasma. This study investigated the potential for the development of chronic SCN^- toxicity in rainbow trout by chronic exposure to CN^-. Juvenile trout were exposed to 8.4 or 25.4 µg CN^-/L, 5.2 or 42.1 mg SCN^-/L for 16 weeks. Growth, histopathology, physiological, and biochemical parameters were measured. Fish exposed to 42.1 mg SCN^-/L has lower final body weights, hepato- and splenosomatic indices, hematocrit, and elevated plasma SCN^- levels. A significant bioaccumulation of SCN^-, as well as alterations in thyroid histology, occurred in trout exposed to waterborne SCN^- or CN^-. Exposure of trout to waterborne CN^- resulted in physiological and histological changes, but no chronic SCN^- toxicity was observed.

INFLUENCE OF EXPOSURE TIME ON TISSUE DISTRIBUTION OF MERCURY IN RAINBOW TROUT.

A.J. Niimi and G.P. Kissoon, Department of Fisheries and Oceans, Great Lakes Laboratory for Fisheries and Aquatic Sciences, Burlington, Ontario.

Subadult rainbow trout (Onchorynchus mykiss) were exposed to four concentrations of waterborne methyl mercury chloride (MMC) and mercuric chloride ((MC) each until death, and concentrations in the gill, liver, spleen, brain, kidney and muscle were determined. The mean time to death among the 20 fish per concentration ranged from 1 day for those exposed to 34 µg/L MMC to 58 days for fish exposed to 3 µg/L. Fish exposed to 426 µg/L MC died after 2 days, and the last fish exposed to 64 µg/L died after 130 days. There were large differences in the number of days required for the 20 fish to die among the concentrations. For example, fish exposed to 34 µg/L MMC all died within 1 day, while fish exposed to 34 µg/L died between 30-98 days.

Mercury concentrations at death were different among the tissues examined. Concentrations were highest among the fish exposed to the lowest concentrations, with the kidney and liver containing mean concentrations in excess of 200 mg/kg MMC. The distribution patterns among tissues were similar to ten adult rainbow trout collected from Lake Ontario whose tissue concentrations of total mercury were below mg/kg levels. There was a linear increase bewteen mercury concentration in liver, spleen, kidney and muscle when concentrations were expressed as exposure concentration x days to death. This response could suggest the mercury concentrations in these tissues may not have been the primary cause of death because the concentrations at death among fish exposed to higher concentrations were considerably lower than fish that died after exposure to lower mercury concentrations. These results could suggest mercury accumulation by trout in these tissues may not have been the primary cause of death.

COPPER AND CADMIUM BINDING TO FISH GILLS: EFFECTS OF COMPETITION AND COMPLEXATION.

R.C. Playle and D.G. Dixon, Department of Biology, University of Waterloo, Waterloo, Ontario.

Adult fathead minnows were exposed to 15 $\mu g \cdot L^{-1}$ Cu for 2-3 h, in synthetic soft water (Ca^{++}, Na^{+} - 50 $\mu eq \cdot L^{-1}$; pH 4.8 or 6.3). Gill Cu concentrations were 1.7 µg $Cu \cdot g^{-1}$ wet weight for both pH 4.8 and 6.3 Cu exposures; background was 0.8 µg $Cu \cdot g^{-1}$. 2100 or 4000 $\mu eq \cdot L^{-1}$ added Ca^{++} reduced gill Cu during exposures at pH 4.8, but not at pH 6.3. EDTA eliminated Cu deposition at both pH 4.8 and 6.3 when equimolar with Cu, but reduced Cu deposition (by 50%) when half equimolar only in the pH 4.8 exposures. These results can be explained by Ca^{++} and H^{+} competition with Cu for gill binding sites, and by complexation of Cu by EDTA. In addition >4 $mg \cdot L^{-1}$ dissolved organic carbon (DOC) also reduced Cu binding to gills, with total protection at 5-6 $mg \cdot L^{-1}$. In Cu and Cd mixtures (15 $\mu g \cdot L^{-1}$ Cu, 6 $\mu g \cdot L^{-1}$ Cd), neither metal reduced binding of the other metal, indicating many gill binding areas or different binding sites for each metal. DOC and EDTA were less effective in preventing Cd binding to carboxyl and other groups on the gills, either through competition or complexation, will reduce toxicological and physiological effects of metals like Cu and Cd.

CADMIUM AND LEAD CONCENTRATIONS OF Nuphar variegatum IN RELATION TO WETLAND TYPE.

E. Thompson, F. Pick and L.I. Bendell-Young, Department of Biology, University of Ottawa, Ottawa, Ontario.

Nuphar variegatum from 5 bogs, 3 mineral poor and 3 minerotrophic fens were analyzed for Pb and Cd in leaves and petioles. Analysis of variance was used to determine if Cd and Pb in the two plant tissues differed among wetlands. Leaf Cd was significantly greater in Nuphar sampled from 4 of 5 bogs vs Nuphar sampled from the mineral-poor and minerotrophic fens ($P < 0.05$). Leaf Pb was greatest in Nuphar sampled from the bog with the lowest alkalinity and pH. Differences in Pb and Cd petioles concentrations were independent of wetland type. Fens undergo a natural acidification process which transform such fens into bogs. Anthropogenic acidification could accelerate this process. Results of this study suggest that acidification of wetlands will result in increased accumulation of Cd in leaves of associated Nuphar populations. This in turn will result in increased amounts of Cd available for accumulation by mammals which rely on Nuphar for food.

TRACE METALS IN SEDIMENTS INFLUENCED BY COPPER MINE TAILINGS.

R.G. Trucco, I. Inda and M.L. Fernández, Universidad Católica del Norte, Faculted de Ciencias del Mar, Coquimbo-Chile.

This study compares the distribution of trace metals in sediments of water systems influenced by copper mine tailings and areas not affected by these effluents.

Sediment samples were collected every 15 days during one year period in 19 stations distributed along Caren Stream (Chile) and their effluents. The top layer of 10 cm was sampled and trace metal treatment was done using acid digestion procedure in duplicate samples (Environment Canada, 1983). Cadmium, copper, manganese and molybdenum concentrations were determined by atomic absorption spectrophotometry using a Shimadzu model AA-670 spectrophotometer coupled with a graphic printer model PR-4.

The results expressed in mg/kg, dry weight, shows that cadmium, copper and manganese was higher in areas not influenced by the tailings. Manganese was associated with organic matter. However, for molybdenum, the maximum values were determined in sediments influenced directly by the tailings. Based on the results alternative explanations for the distribution of the metals are discussed. In Chile there are no guidelines for contaminant levels in sediments.

EVALUATION DE LA TOXICITÉ ENVIRONNEMENTALE PAR DES INDICES BIOCHEIMIQUES DE CROISSANCE.

E. Mayrand et J. Pellerin, Départmente d'Océanographique, UQAR, Rimouski, Québec.

Cette étude a pour de déterminer si l'effet d'une toxicité anthropogénique sur la faune marine peut être décelée au niveau des variations des réponses biochimiques.

L'étude porte sur un Bivalve de l'estuaire du St-Laurent, Mya arenario. Les indices de croissance ARN:ADN, protéines:ADN et protéines:ARN sont utilisés pour trols organes. Nous avons d'abord évalué la variabilité naturelle de ces indices, à petite échelle. L'homogénété des indices est conservée pour des individus de 12 à 18 ans et de 50 à 69 mm de longueur. La taille des quadrats d'échantillonnage doit être limitée à 6.25 m^2 pour réduire la variabilité spatiale des indices. En respectant ces contraintes, des transferts réciproques de myes ont ensuite été effectués entre un site sain (BIC) et un site pollué (Rimouski). Les animaux, locaux et transférés, ont été récoltés après 6 mois. Les résultats de cette expérience seront présentés.

HEPATIC BIOCHEMICAL OBJECTIVES TO PROVIDE SAFE LEVELS FOR RAINBOW TROUT.

C.J.P. McKean and J. Deniseger, Ministry of Environment, Water Quality Branch, British Columbia, and Ministry of Environment, Environmental Protection, Nanaimo, British Columbia.

The Ministry of Environment has been experimenting with biochemical techniques to determine safe metal levels for rainbow trout. Buttle Lake received extensive metal loading from 1967-1985 from a copper/lead/zinc mine resulting in significant reduction in the fisheries production. Roch and McCarter (1982) developed a rainbow trout metallothionein objective for Buttle Lake using long-term bioassays. Since 1985, reclaimation of the mine site has significantly reduced metal input to the lake. The result has been a reduction in the metallothionein concentrations below the objective and an increase in the fisheries production of the system. The Ministry is presently using long-term bioassays to establish metallothionein and other biochemical objectives to protect the fisheries resources of the Taolum River. Acid mine drainage from an abandoned copper mine in the headwaters has eliminated a 40,000 pink salmon run, 500 coho run, plus steelhead, and cutthroat trout from the river.

ON SITE TREATMENT OF ST. MARYS RIVER SEDIMENTS. T. Murphy, K. McCabe and M. Fox, Environment Canada, Lakes Research Branch, National Water Research Institute, Burlington, Ontario.

Sediments near Bellview Marine Park on the St. Marys River are moderately contaminated. The concentration of chlorinated hydrocarbons is near background values. The concentrations of PCBs is low. Although the concentrations of metals and PAHs are moderate, studies have shown that the benthic community in the proposed treatment site is severely impaired. No aquatic plants or mayflies were observed, and oil and grease exceed 1.5%.

Laboratory bioassays of sediments collected at this site this past winter were not toxic to Daphnia (waterflea), Photobacterium, or lettuce. However, these sediments were toxic in the summer. Natural processes in winter such as oxidation, suppress the toxicity. The significance of the seasonal change in toxicity will be presented relative to the potential for treatment.

COAL TAR CONTAMINATION OF HAMILTON HARBOUR. T. Murphy, A. Moller, M. Fox, E. Nagy and M.S. Burgham, Environment Canada, National Water Research Institute, Burlington, Ontario.

To support the remedial action plan of Hamilton Harbour, and to determine the extent of coal tar contamination in a toxic area of the harbour, 81 sediment cores were collected for chemical and biological study. Approximately 55,000 m^3 of sediments bound by Randle Reef, pier 15, and Stelco are contaminated with coal tar. The coal tar distribution is variable but the highest concentrations are near the Stelco outfall pipes and the Hamilton-Wentworth combined sewer outfall pipe. The total concentration of the 16 polynuclear aromatic hydrocacrbons (PAHs) in 48,300 m^3 of near-shore sediments exceeds 200 µg/g. The concentration of PAHs that results in the death of 50% of Daphnia magna and Hexagenia is less than 244 µg/g and 329 µg/g, respectively. Sediments containing more than 89 µg/g of PAHs suppress at least half of the photoactivity of Photobacterium phosphoreum. The acute toxicity of the sediments of all of Hamilton Harbour is significantly correlated to the PAH concentration.

THE ROLE OF SEDIMENT pH AND REDOX POTENTIAL IN DETERMINING METAL BIOAVAILABILITY TO ISOETID MACROPHYTES. L.J. Jackson, J. Kalff and J.B. Rasmussen, Department of Biology, McGill University, Montréal, Québec.

We investigated the role of sediment pH and redox potential in determining the bioavailability of Al, Cu, Fe, Mn and Zn to isoetid macrophytes. Regression models related plant metal concentrations to underlying sediment metal concentrations and physicochemical attributes. Log sediment metal accounted for 22-79% of the variation of the log of plant metal; an additional 5-45% variation was explained by pH. Sediment redox potential > 200 mV greatly decreased metal availability. Acidification of moderately reduced sediments may result in increases in isoetid metal levels. These results point to the role of rooted aquatic plants in the transfer of sediment bound metals to herbivores.

CAN PLANT TOXIC COMPOUNDS AFFECT AQUATIC ORGANISMS? A.M. Zobel and C. Santos, Department of Chemistry, Trent University, Peterborough, Ontario.

Dead tissue of trees (e.g. Populus), growing in great concentration close to a small lake, can be a threat to aquatic organisms because of the release into the water of potentially toxic phenolic compounds, which act as natural plant protectors. It has been shown in this laboratory (Zobel and Brown, 1990, J. Chem. Ecol. 90: 693) that many plants belonging to the Umbelliferae and Rutaceace contain high concentrations of furanocoumarins on the surface - up to mg/g fresh weight. By simulating rain or dipping in water at room temperature were removed from the plant surface hundreds of micrograms of furanocumarins per gram fresh weight. These compounds are known to cause photophytodermatitus by reaction with DNA after ultraviolet irradiation. Such concentrations inhibit growth of some stains of bacteria and division of embryonic cells. Histochemical observations of diploid and triploid trout eggs and embryos after the action of furanocumarins revealed changes in structure of eu- and heterochromatin in the interphase nuclei, and chorosomal aberrations visible during mitosis. As furanocumarins are well-known as fish poisons, the possibility should be entertained of their removal from the plant surface into the water.

BEST STUDENT AWARD/PRIX POUR LE MEILLEURE ÉTUDIANT

Awards for the Best Student Platform and Poster Presentations are presented annually by the Workshop Organizing Committee to encourage student participation. The 1991 winners were:

Best Student Platform Presentation

Mary H. Murdoch
Department of Zoology, University of Guelph

Best Student Poster Presentation

Claire Vanier
Départmente de Biologie, Université du Québec à Montréal

LIST OF AUTHORS/LISTE DES AUTEURS

Pages in bold indicate senior author

Author	Page
Abernethy, S.G.	**166**
Adams, S.M.	123,187
Addison, R.F.	**189,311**
Allard, G.	**13**
Amyot, M.	**67**
Agger, C.T.	280
Audet, C.	128
Barrie, L.A.	**305**
Baumann, P.C.	**131**
Becker, P.R.	**287**
Béland, P.	350
Bendell-Young, L.I.	354
Bennie, D.T.	183
Bentzen, E.	**102**
Bergman, Å.	**259**
Bermingham, N.	**185**
Biddinger, G.R.	**239**
Bidleman, T.F.	**253**
Billeck, B.N.	264
Birkholz, D.A.	351
Biseth, A.	312
Black, M.C.	187
Blaise, C.	184,184
Boston, H.L.	187
Brandes, R.	**220**
Brown, K.R.	340,345
Bruneau, S.	265
Brunskill. G.J.	264
Bureau, J.	123
Burgham, M.S.	356
Burnison, B.K.	36,183
Campbell, P.G.C.	13,325
Capel, M.	35
Careau, H.	**299**
Carey, J.H.	**183**
Cashore, A.	139
Casillas, E.	198
Caux, P.-Y.	**352**
Chant, L.A.	37
Chapman, P.M.	**2**
Charlton, M.N.	**36**
Chernyak, S.M.	269,309,310
Clarke-Whistler, K.	**218**
Collier, T.K.	198
Cornett, R.J.	**37**
Costan, G.	**183**,185
Couillard, Y.	**325**
Couture, P.	**184**
Craig, G.R.	**185,219**
Currie, R.S.	350
Cuthbert, I.D.	**87**
Day, K.E.	**235,243**
Decho, A.W.	26
Delille, D.	134
Deniseger, J.	355
Desgranges, J.L.	161
Dewailly, E.	**265**,299
Dietz, R.	**280**
Dillon, T.	**242**
Dixon, D.G.	314,352,353
Doe, K.G.	222
Drake, D.C.	104
Edge, T.	**238**
Evans, R.D.	81,317
Fairchild, W.L.	**350**
Ferguson, M.L.	22
Fernández, M.L.	354
Fisher, N.S.	26
Ford, C.A.	312
Ford, J.	292,296
Fournier, R.	**82**
Fox, M.E.	36,356,356
Fulthorpe, R.	139
Gagnon, C.	**27**
Gatz, A.J.	187
Gauvin, D.	299
Gingras, S.	265
Glahder, C.M.	**272**
Gobas, F.A.P.C.	161
Grapentine, L.C.	**141**
Greeley, Jr., M.S.	187
Gregor, D.J.	**308**
Griffiths, R.W.	148
Grover, R.	138
Guay, I.	**200**
Gubala, C.P.	**292,311**
Haffner, G.D.	**161**
Halbrook, R.S.	**187**
Haraguchi, K.	259
Hart, D.	219
Hauschield, C.	102
Hayes, M.A.	106
Hebert, P.D.N.	117
Hesslein, R.	35
van den Heuvel, M.R.	123
Hickie, B.E.	**350**
Hill, W.R.	187
Hinckley, D.	269
Hinman, M.L.	239
Hinzman, R.L.	187
Hodson, P.V.	123,313,350,351
Hopkinson, R.	138
Howard, D.E.	**317**
Huebert, D.B.	**86,144**
Hughes, J.S.	**169**
Inda, I.	354
Jaagumagi, R.	**242**
Jackson, D.A.	63
Jackson, L.J.	**357**
Jenkins, K.J.	**61**
Johnson, L.L.	198
Kalff, J.	357
Karau, J.	243
Kaushik, N.	148
Kemper, J.B.	**219**
Kennedy, S.	123
Kerr, L.	138
Kirby, G.M.	**106**
Kissoon, G.P.	353
Knuth, M.L.	**137**
Koenig, B.G.	22
Kolobova, T.	269
Van Der Kraak, G.J.	190
Krynitsky, A.	269
Kuroki, H.	259
Laliberté, C.	265
Landers, D.H.	**292,296**,311
Langston, W.J.	**81**
Lanno, R.P.	**352**
Lapierre, L.	128
Lasenby, D.C.	**81**
Lau, Y.L.	**15**

Author	Page
Lean, D.R.S.	102,102
Lockhart, W.L.	123,264
Luoma, S.N.	26
Macdonald, C.R.	22,101
MacDonald, D.D.	241
Mackay, D.	245,350
Mackenzie, I.	219
Maheu, S.	49
Malley, D.F.	86
Marsot, P.	82
Martel, P.	123
Mason, A.Z.	61
Matheson, R.A.F.	238
Mayrand, E.	325,355
McCabe, K.	356
McCarthy, J.F.	187
McCormick, J.H.	133
McIntyre, T.	238
McKean, C.J.P.	353
McLeay, D.J.	222
McMaster, M.E.	190
McNaught, D.C.	104
McPherson, R.G.	340,345
Melnikov, S.	308
Ménard, L.	184
Metcalfe, C.D.	22,101
Metner, D.A.	123,264
Meyers, M.S.	198
Mierle, G.	65
Miller, J.	123,218
Milton, C.M.	37
Moller, A.	356
Monetti, M.	311
Morin, B.	128
Mucci, A.	27
Muir, D.C.G.	139,253,312,350
Munkittrick, K.R.	123,190
Murdoch, M.H.	117
Murphy, T.	356,356
Nagy, E.	356
Nakatsu, C.	139
Nantel, A.	265
Ng, J.	139
Niimi, A.J.	353
Norstrom, R.J.	259,311,311
Noton, L.R.	219
Oehme, M.	312
Orr, P.	219
Paine, J.F.	123
Paniv, G.V.	309
Parker, W.R.	163
Parrott, J.L.	314
Pauwels, S.J.	239
Peel, M.	139
Pellerin, J.	120,355
Pellerin-Massicotte, J.	325
Pelletier, E.	27,39,49,82, 134,161,330,351
Peters, R.H.	98
Peterson, M.J.	197
Pick, F.	354
Pinel-Alloul, B.	67
Porebski, L.M.	222,243
Portt, C.B.	190
Planas, D.	349
Playle, R.C.	353
Rasmussen, J.B.	101,357
Razurel, E.	161
Reinfelder, J.	26
Reynoldson, T.B.	235,243
Rice, C.P.	269
Richer, G.	98
Rouleau, C.	39,330
Rowan, D.J.	101
Roy, Y.	185
Ryon, M.G.	187
St.-Laurent, D.	184
St.-Louis, R.	82
St.-Pierre, C.	325
Saint-Marie, B.	351
Santos, C.	357
Savinova, T.N.	310
Scarratt, D.J.	84
Scheider, W.A.	102
Schilling, E.M.	187
Schlabach, M.	312
Scroggins, R.P.	163,219
Segstro, M.D. 105	139,311
Servos, M.R. P14	139,314
Shaw, M.	86
Shay, J.M.	144
Simon, M.	312
Simon, R.	134
Singer, M.M.	175
Smalheer, D.L.	175
Smith, I.R.	123,190
Smith, J.G.	187
Smith, S.L.	241
Solomon, K.	148
Somers, K.M.	63
Southworth, G.R.	187
Spry, D.J.	214
Stegman, J.J.	123
Stein, J.E.	198
Stewart, A.J.	187
Stewart,R.	278
Swanson, S.M.	351
Szilagyi, F.	139
Tessier, A.	325
Thibodeau, M.	86
Thomas, R.	296,311
Thompson, E.	354
Tjälve, H.	39,330
Tjeedema, R.S.	175
Tramblay, R.	120
Trew, D.O.	219
Trucco, R.G	354
Tysban, A.V.	309
Vanier, C.	349
Varanasi, U.	198
Vézina, A.	299
Wagemann, R.	35,278
Wainman, B.C.	102
Waite, D.T.	138
Walla, M.D.	253
Walsh, G.	128
Wania, F.	245
Warren, L.A.	37
Weber, J.P.	265
Webster, G.R.B.	139
Wise, S.A.	287
Wyndham, R.C.	139
Yan, N.D.	81
Yarechewski, A.L.	350
Yee, S.G.	222
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ADDENDUM/ADDENDA

The 16th Annual Aquatic Toxicity Workshop was held on November 6-8, 1989, in Winnipeg, Manitoba. A Proceedings from that Workshop was not published because of technical difficulties. The following two papers represented some of the contributions at that Workshop.

LONG-TERM EFFECTS OF AN ACUTE ACID PULSE ON JUVENILE BLACK CRAPPIE, POMOXIS NIGROMACULATUS

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ABSTRACT

Gill chloride cell proliferation accompanied a sharp decrease in blood osmolality in juvenile black crappie, Pomoxis nigromaculatus, exposed for 12 days to soft water at pH 4.0. When the pH was restored to 7.0 blood osmolality and chloride cell numbers returned to near pre-exposure values, but condition factor remained low for the entire 30 day recovery period.

SUMMARY

In addition to slow acidification of watersheds in regions with acid precipitation, there are times when pulses of water at extremely low pH are delivered to lakes and streams, most obviously during spring snowmelts but also during other parts of the year (Reader and Dempsy, 1989). These acid pulses can sometimes result in fish kills, but usually the fish survive them. In the present experiment we wanted to determine if there are long-term sublethal effects of short-term, near-lethal acid pulses.

Juvenile black crappie (ca 4-9 g) were transferred from holding tanks with Lake Superior water (hardness of 45 mg/L as $CaCO_3$) to replicate exposure tanks receiving diluted Lake Superior water (hardness of 4 mg/L) at 20°C. They were fed rinsed brine shrimp once (weekends) or twice (weekdays) daily. The dilution water contained (mg/L): Ca 1.0, Na 0.3, Cl 0.3, Al <0.002. The test fish were acclimated to this water at pH 7.0 for 10 days before sulfuric acid was gradually metered to the flow-through system. Duration of exposure to the experimental pH, 4.0, was based on the time needed for blood osmolality in subsamples of fish to fall to the near-lethal threshold of about 200 mosmol/kg. Acid delivery was then stopped and the pH returned to 7.0. Samples of fish (N=8-11 except for 30 day recovery where N=4) were preserved for histological study at the time of distribution to the test tanks, after acclimation in dilution water, after the blood osmolality threshold value had been reached at pH 4.0, and at 5, 15, and 30 days after restoration of pH to 7.0. See McCormick et al. (1989) for further details on the exposure regimen and water chemistry and Leino et al. (1984, 1990) for methods regarding the histological and morphometrical analysis of the gills.

Exposure of black crappie to pH 4.0 for 12 days resulted in a loss of blood osmolality from about 283 mosmol/kg to about 200 mosmol/kg (Fig. 1, also McCormick et al. 1989). When the pH was restored to 7.0, blood osmolality recovered to near, but not quite, original values--about 269 mosmol/kg--within 30 days (Fig. 1). While no obvious gill pathology was observed, a marked proliferation of chloride (ionoregulatory) cells accompanied the acid-associated fall in blood osmolality (Fig. 2). The number of chloride cells returned to pre-exposure values within 5 days after restoration of pH to 7.0 (Fig. 2). However, growth ceased and condition factor[a] declined and remained low (0.89 vs. 1.02 pre-exposure value, Fig. 3) even after 30 days of recovery. Therefore, recovery from an acid pulse may be energetically expensive and detrimental to the condition of young black crappie even though the morphological appearance of the gill ionoregulatory tissue rapidly returns to normal. In northern climates, body size may greatly affect overwintering survival of young fish (e.g., Oliver et al. 1979; Johnson and Evans 1990; Shuter and Ihssen 1991). The present experiment suggests that, in some instances, acid pulses could contribute to poor condition of young-of-the-year fish.

[a] condition factor=K=Wg • 10^5/Lmm^3

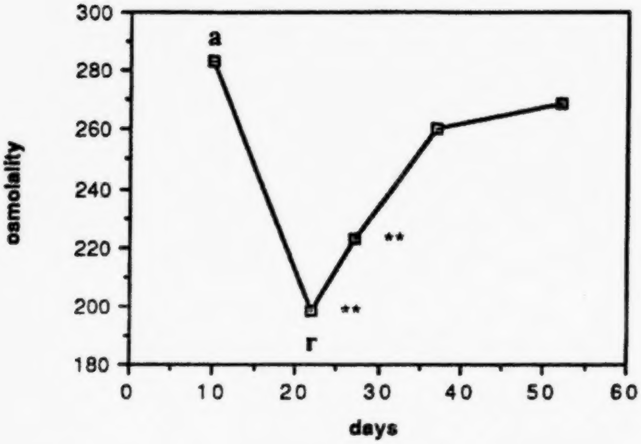

Fig. 1. Mean blood osmolality (mosmol/kg) of juvenile black crappie exposed for 12 days to pH 4.0 (after McCormick et al. 1989); pH brought to 4.0 at point a and returned to pH 7.0 at point r. ANOVA revealed significant treatment effects, p<0.001. **=significantly different from pH 7.0 pre-exposure value (a) with Tukey's test, p<0.05.

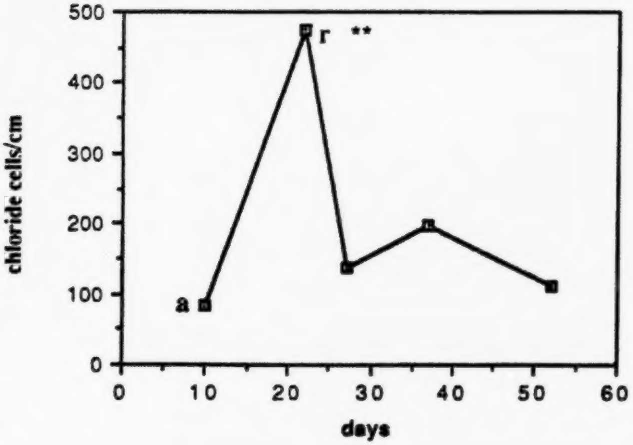

Fig. 2. Mean gill chloride cell numbers in juveniles exposed for 12 days to pH 4.0. ANOVA revealed significant treatment effects, p<0.001. **=significantly different from pH 7.0 (a) with Tukey's test, p<0.05.

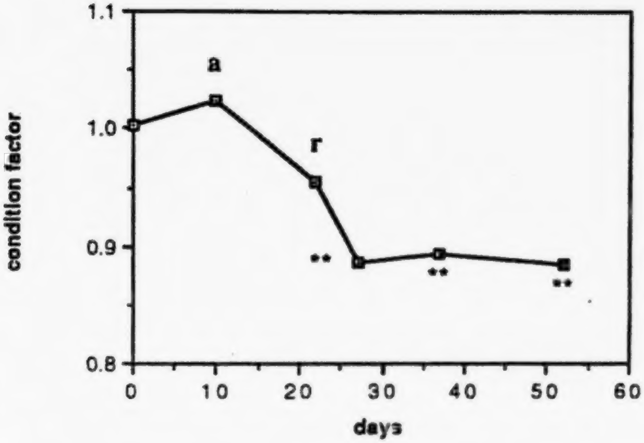

Fig. 3. Mean condition factor of juveniles exposed for 12 days to pH 4.0. ANOVA revealed significant treatment effects, p=0.003. **=significantly different from pH 7.0 (a) with Tukey's test, p<0.05.

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THE EFFECTS OF BOREAL SUBARCTIC VERY-SOFT WATERS AND URANIUM EFFLUENTS ON SOME POPULATION CHARACTERISTICS OF Ceriodaphnia dubia (CRUSTACEA: CLADOCERA)

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ABSTRACT

Seven-day Ceriodaphnia dubia bioassays were used to examine two very soft dilute waters and two uranium effluents, including dilutions, from northern Saskatchewan. Both soft and moderately hard culture waters were used to dilute the effluents. Population symptoms of chronic stress occurred in the soft waters as well as the effluents. Levels of survival and or reproduction were low except in moderately hard water. Bioassay animals had been released into the soft waters as neonates. Either Ceriodaphnia dubia needs to be cultured for at least several generations in very soft dilute waters, or it does not tolerate such waters therefore it is not suitable for use in bioassays when dilution waters are very soft.

INTRODUCTION

Northern lakes and rivers are generally regarded as being especially vulnerable to ecological damage during development, because they are fragile ecosystems in a harsh environment (Kwiatkowski et al., 1987). Uranium exploration, mining and milling activities have been extensive in the subarctic Precambrian Shield area of northern Saskatchewan (Melville, 1989b). These activities can cause severe ecological damage to aquatic systems (Abouguendia et al., 1983). Mill effluents in particular can cause substantial changes in water quality (Hynes et al., 1987).

In this paper I report the effects of two uranium effluents on some population characteristics of Ceriodaphnia dubia in bioassay experiments. Ceriodaphnia dubia, a tiny shrimp-like organism, is a standard test organism (US EPA, 1985). Bioassays are used to determine the effects of effluents and other substances on the environment, establish water quality criteria and ultimately protect aquatic systems from the introduction of toxic substances (Cowgill, 1987). The information in this paper will help us assess the effects of uranium milling on northern lake ecosystems.

In addition, the effects of two very soft dilute waters from the boreal subarctic northern Saskatchewan are presented. Northern waters are often very soft and dilute (Abouguendia et al., 1983), unlike most more southerly waters, thus they could have an effect on Ceriodaphnia. Previous work (Melville, unpublished data) indicated that hardness over a wide range of values, but exclusive of very soft waters, had no effect on unacclimatized Ceriodaphnia.

METHODS

Treatments were done as 7 day "life-cycle" bioassays (Mount and Norberg, 1984). Treatments were done over two experiments, rather than a single experiment, for logistical reasons. In the first experiment (B), the two effluents were examined, as well as the two northern waters. Each effluent was also diluted to a range of concentrations using the northern water from the nearest site. The second experiment (A) duplicated the first, except that harder pond culture water was tested and used as the dilution water for the effluents.

Experimental variables are presented in Table 1. Temperature was measured using a hand-held thermometer. Light intensity and dissolved oxygen were measured using commercial electronic meters. The Saskatchewan Research Council analytical laboratory analyzed water and effluent samples for a large suite of constituents using standard methods (APHA, 1976), as well as inductively coupled plasma atomic emissions spectroscopy.

Algal species used as food were mass cultured according to methods in Taub and Kindig (1988). Algal counts were made by way of a hemacytometer and compound microscope.

The C. dubia were from cultures originally started with animals obtained from the US EPA in Duluth, MN. Body lengths were measured using a stereo microscope fitted with an ocular micrometer.

Sokal and Rohlf (1969) and Hamilton *et al*. (1977, 1978) were used as statistical references.

RESULTS

General water and undiluted effluent quality characteristics are summarized in Table 2.

Survival in high soft water-low effluent mixtures was higher than in the very soft waters (Fig. 1). Above the 25% effluent concentration, survival decreased dramatically, to levels well below survival in the soft waters. In contrast, survival decreased at all effluent concentrations when moderately hard water was used to dilute the effluents (Fig. 1). In this experiment, the LC 50's were 80.8 and 83.7% for sites 1 and 2 respectively. Survival in the harder water was 100%.

Reproduction was far greater in moderately hard water and harder water-low effluent mixtures than in soft water and other effluent mixtures (Fig. 2). This difference in magnitude not withstanding, reproductive trends in soft water and soft water-effluent mixtures are similar to survival patterns in the same media. Reproduction in these media was highest at low-to-intermediate effluent concentrations (Fig. 2). In moderately hard water-effluent mixtures, reproduction declined as effluent concentrations increased (Fig. 2).

Body sizes (Table 3) were smaller in soft dilute waters than in moderately hard water (P's <0.025, Wilcoxon two-sample tests). Body sizes were also smaller in effluent mixtures, in most cases decreasing as the effluent concentrations increased (Table 3).

Many elemental constituents of the waters and effluents are listed in Appendix 1. Elevated levels of three metals in particular: Cr, Pb and Zn, occurred in the soft waters. The effluents all contained a number of metals at elevated concentrations.

DISCUSSION

Clearly, population symptoms of chronic stress occurred in *Ceriodaphnia dubia* in the very soft dilute northern waters, as well as in effluent dilutions. Mortality exceeded 10%, little or no reproduction occurred, and body sizes, hence growth rates, were less than those in moderately hard water. Perhaps *Ceriodaphnia dubia* cannot osmo/iono-

Table 1. Experimental variables.

Variable	Units	Value
Temperature	°C	22.5 - 25.4
Light intensity	lux	160 - 195
Photoperiod	h light/h dark	16/8
Dissolved O_2	mgl^{-1}	6.2 - 8.0
Feeding rate	cells $ml^{-1}d^{-1}$	
Chlamydomonas reinhardtii		1.4×10^5
Chlorella pyrenoidsoa		2.0×10^5
Selenastrum capricornutum		0.6×10^5
Feeding frequency	d^{-1}	2
Water volume	ml	15
Ceriodaphnia dubia density	vessel $^{-1}$	1
Replicates/treatment (includes dilutions)		10
C. dubia starting ages	h	< 5

Table 2. General water quality characteristics of the waters and effluents examined. P = pond (culture) water, E = effluent, S = site specific soft water, 1,2 = sites, A,B = experiments.

Characteristic	Treatment water/effluent type						
	P*A	E1A	E2A	S1B	E1B	S2B	E2B
Hardness as $CaCo_3$	150	1670	1580	8.6	2120	5.9	1470
Conductivity umhos cm^{-1}	525	2680	4570	38	3430	28	4580
pH	8.0	6.1	7.0	6.4	6.1	6.8	7.1

*Melville and Richert (in press)

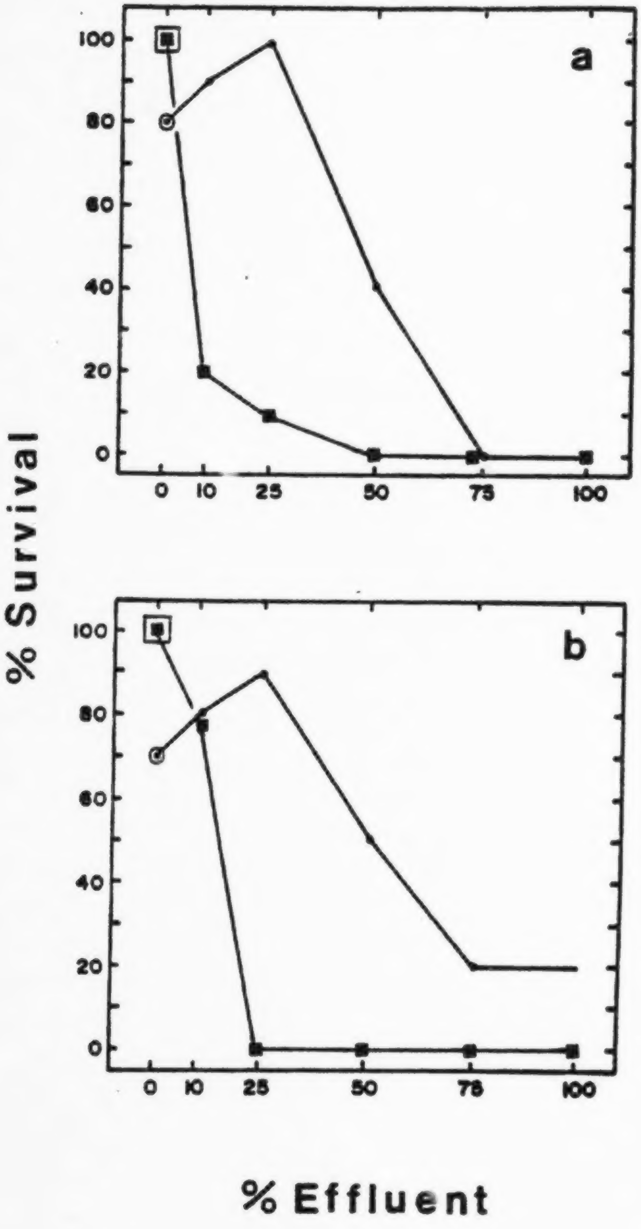

Figure 1. Survival of Ceriodaphnia dubia. a. in soft water and effluent from site 1. b. in soft water and effluent from site 2. circles and squares = effluents, experiments A and B respectively. concentric circles and squares = soft and culture waters respectively.

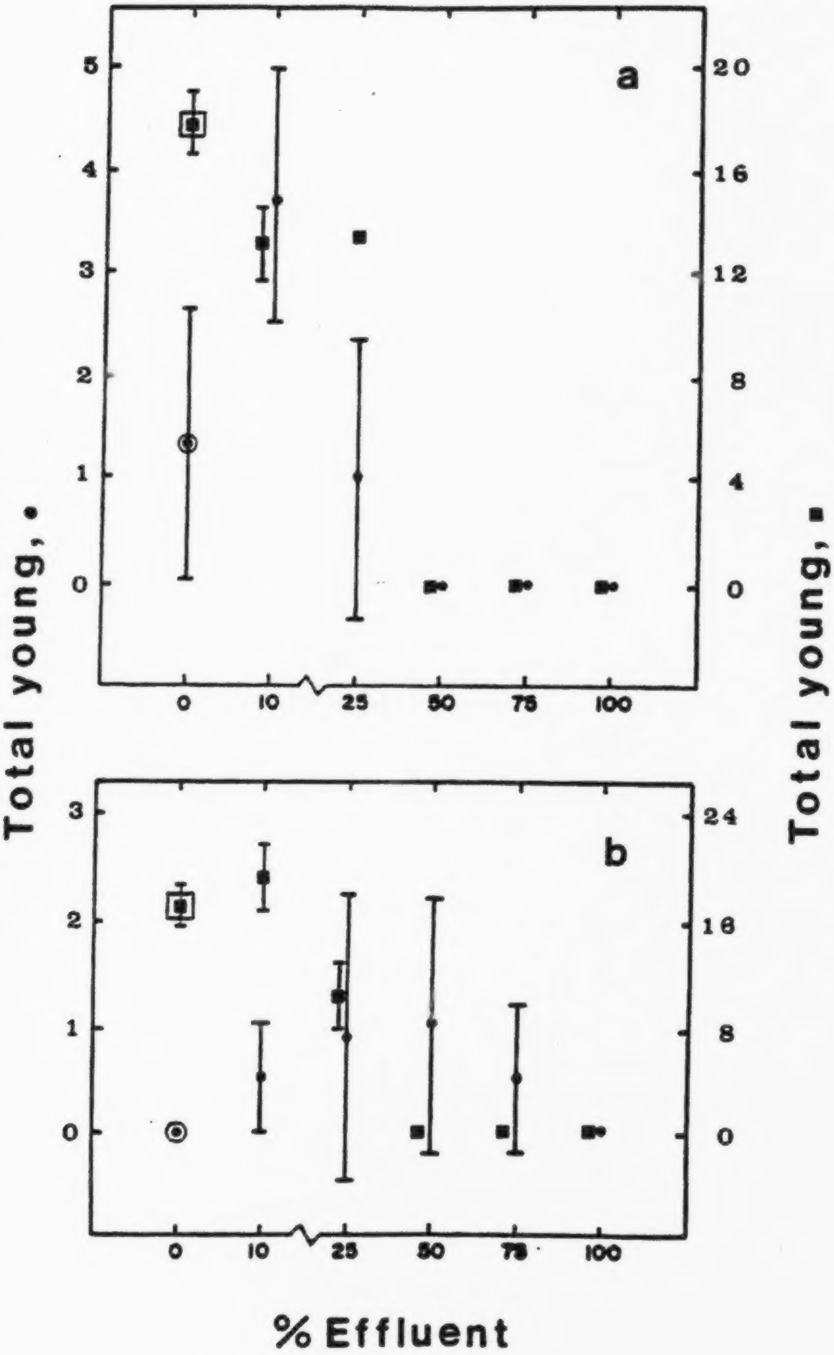

Figure 2. Mean total number of young produced by Ceriodaphnia dubia over 3 broods. Vertical bars = 1 Std. Dev. a,b, symbols as in Figure 1.

Table 3. Body lengths of (surviving) *Ceriodaphnia* after 7 days of treatment. Treatment codes as in Table 2.

Treatment water /effluent type		Mean length (mm)	Std. Dev. (mm)	Number
PA		1.08	0.01	6
E1A	10%	0.92	0.08	2
	25%	0.96	--	1
E2A	10%	1.11	0.05	4
	25%	0.90	--	1
S1B		0.93	0.01	3
E1B	10%	0.91	0.05	3
	25%	0.86	0	2
	50%	0.58	0.07	3
S2B		0.82	0.05	3
E2B	10%	0.82	0.05	4
	25%	0.81	0.05	3
	50%	0.74	0.07	3
	75%	0.68	--	1
	100%	0.64	0	2

regulate and or molt properly in very soft dilute waters. The trace elements Cr, Pb and Zn also occurred at concentrations harmful to aquatic fauna (CCREM, 1987), although metals such as Cr occur naturally at elevated levels in northern systems containing 'typical' zooplankton communities (e.g., Melville, 1989a). Other experimental conditions were conducive to good health in *Ceriodaphnia*, including the diet (Melville and Richert, in press; also Melville, unpublished data).

The addition of salts in the low effluent concentrations probably alleviated the stress somewhat of the very soft dilute waters, since survival improved. These salts could also have counteracted, in part, any effects of harmful levels of Cr, Pb and Zn. However, mitigative effects of effluent salts would have been relatively small, since reproduction remained very low. Each effluent also contained at least several metals at toxic concentrations (CCREM, 1987). Mitigation by way of moderate amounts of effluent salts is not a wholly satisfactory explanation, because *Ceriodaphnia* survival levels were indicative of stress for all moderately hard water-effluent mixtures (Fig. 1).

Ceriodaphnia *dubia* used in these experiments were not acclimatized to non-culture media before being introduced into them. This was not done, because *C. dubia* can readily survive, grow and reproduce normally, after being transferred to media differing in conductance from source media by as much as 1500 umhos/cm (Melville, unpublished data). They may not be able to handle such manipulations when very soft waters are involved. This possibility is currently under investigation. Either *Ceriodaphnia* *dubia* needs to be cultured for at least several generations in very soft dilute waters, or it does not tolerate such waters therefore it is not suitable for use in bioassays when dilution waters are very soft.

ACKNOWLEDGEMENTS

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Appendix 1. Some major and trace constituents of the waters and effluents tested. The units are mgl^{-1}. Treatment codes as in Table 2.

Constituent	Treatment water/effluent type						
	P*A	E1A	E2A	S1B	E1B	S2B	E2B
Ca	48	638	556	15	829	12.0	511
K	4.4	20	56	2.0	21	1.6	57
Mg	20	17	47	1.8	13	1.2	46
Na	42	32	472	24	28	19	478
Cl	--	15	708	15	26	12	745
HCO_3	--	7.0	27	0.7	21	0.5	57
SO_4	--	1740	1680	9.0	2240	8.0	1540
Ag	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	0.063	<0.005	0.13	<0.001	<0.005	<0.001	<0.052
As	--	--	--	<0.001	<0.081	0.001	0.160
B	<0.05	0.32	0.17	0.05	0.38	0.05	0.10
Ba	0.043	0.062	0.019	<0.001	0.058	<0.001	<0.037
Be	<0.001	<0.001	0.001	0.002	<0.001	<0.001	<0.001
Cd	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.001
Co	<0.001	<0.001	0.005	<0.001	0.006	0.001	<0.001
Cr	<0.001	0.011	0.014	0.031	0.023	0.029	0.026
Cu	0.004	0.002	0.005	<0.001	0.002	<0.001	<0.001
Fe	0.017	0.180	0.110	6.10	0.450	1.10	0.200
Mn	<0.001	0.024	0.120	0.200	0.035	0.035	0.180
Mo	<0.005	0.009	6.30	0.096	0.140	0.110	5.80
Ni	0.002	0.200	0.065	<0.001	0.330	<0.001	0.100
P	0.52	0.16	0.13	<0.05	0.06	<0.05	0.17
Pb	<0.005	0.009	<0.005	0.034	0.027	0.025	0.018
Se	<0.001	0.020	0.005	<0.001	0.015	<0.001	0.006
Si (soluable)	<0.2	<0.2	1.1	2.3	<0.2	1.5	1.3
Ti	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
U	--	0.008	0.530	<0.005	0.001	<0.005	0.630
V	<0.01	<0.01	0.02	0.09	0.06	0.08	0.08
W	<0.005	<0.005	0.006	<0.005	<0.005	<0.005	<0.005
Zn	0.017	0.150	0.014	0.140	0.010	0.027	<0.001

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

The Proceedings of the Annual Aquatic Toxicity Workshops have been published as a series of technical reports listed below. Copies of recent Proceedings may be available from A. Niimi, Continuity Chairman, Aquatic Toxicity Workshop, Department of Fisheries and Oceans, Canada Centre for Inland Waters, Burlington, Ontario L7R 4A6. Copies of most Proceedings are available for a charge from Micromedia Limited, 165 Hotel de Ville, Place du Portage, Hull, Quebec J8X 3X2 (819-770-9928). Their catalog numbers (MLCN) are listed below where applicable.

Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, B.C. Edited by P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuk and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p. (MCLN: 91-06176).

Proceedings of the Fifteenth Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p. (MCLN: 90-01805).

Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p. (MLCN: 88-04587)

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